Journal of
ARACHNOLOGY

PUBLISHED BY THE AMERICAN ARACHNOLOGICAL SOCIETY

JOURNAL OF ARACHNOLOGY

The *Journal of Arachnology* (ISSN 0161-8202), a publication devoted to the study of Arachnida, is published three times each year by *The American Arachnological Society*. **Memberships (yearly):** Membership is open to all those interested in Arachnida. A subscription to the *Journal of Arachnology* and annual meeting notices are included with membership in the Society. Regular, $55; Students, $30; Institutional, $125. Inquiries should be directed to the Membership Secretary (see below). **Back Issues:** James Carrel, 209 Tucker Hall, Missouri University, Columbia, Missouri 65211-7400 USA. Telephone: (573) 882-3037. **Undelivered Issues:** Allen Press, Inc., 810 E. 10th Street, P.O. Box 368, Lawrence, Kansas 66044 USA.

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Cover photo: Female *Phidippus regius* eating a Cuban treefrog (*Osteopilus septentrionalis*) in Lake Placid, Florida (see page 238). Photograph by Martin Fisher.
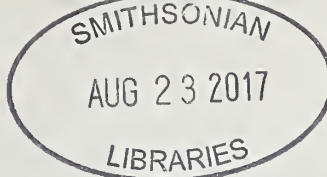
How orb-weavers find and grasp silk lines

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Abstract. Spiders are effectively blind with respect to the lines in their own webs. Species in four orb-weaving families solved the problem of finding lines by tapping with their anterior legs, like a blind man with his cane, and then "following" these anterior legs with more posterior legs, which grasp lines that the anterior legs are already holding. Following behavior occurs during several stages of orb construction, and probably economizes on searching time and effort. The movements of following legs are finely adjusted to the morphological details of the grasping structures on their tarsi (the middle claw and its associated serrate accessory setae): the small searching movements made by following legs have consistent orientations: legs I and II move prolaterally, while legs III and IV moved retrolaterally. These orientations are appropriate to bring the asymmetrically placed middle claw and accessory setae into contact with the line. Spiders solved the additional problem of grasping lines that are more or less parallel to the long axis of the leg by using a previously unrecognized movement; they rotate the distal portion of the leg on its longitudinal axis, orienting the middle claw so that it is more or less perpendicular to the line. As an orb-weaver moves across her web, she probably constantly adjusts the rotation of each leg to align its middle claw perpendicular to the lines that it grasps.

Keywords: Middle tarsal claw, accessory setae, leg movements, web construction behavior

An orb web spider confronts special problems in finding and grasping lines as it moves on its web, because orb-weavers are often active at night and are in any case effectively blind with respect to the lines under which they are walking. Their eyes are probably not capable of resolving such fine objects (Barth 2002), as well as oriented inappropriately, being directed dorsally and laterally, while the web lines are usually ventral to her body. The spider instead relies on her legs (usually legs I and II) to locate new lines. This paper concerns behavioral solutions to these problems.

An animal's behavior always depends on its morphology, so a convenient place to begin is the tarsal morphology involved in grasping web lines. It was established long ago that modifications of the tarsal claws and the serrate accessory setae enable spiders with three tarsal claws to firmly grasp and then release single silk lines (Nielsen 1931; Wilson 1962; Foelix 1970, 2011). Observations with both a dissecting microscope (Nielsen 1931; Wilson 1962) and a scanning electron microscope (Foelix 2011) showed that the middle claw is lowered (as a unit with the lateral claws) to trap the line against the teeth on one (or perhaps more) of the stiff but nevertheless somewhat flexible serrate setae nearby (Fig. 1), where it is squeezed between the ventral surface of the claw and the deflected setae (I will call this "grasping" in the descriptions below). When the line is bent sharply between the serrated accessory setae and the middle claw, the spider's grip is prevented from slipping. When the middle claw is lifted slightly, the grip on the silk is loosened, and the line can slide under the middle claw (Wilson 1962). When the claw is lifted completely, the grip on the line is released: the tension on the line itself, along with the straightening movements of the setae, are thought to propel the line away from the claw (Nielsen 1931). This interpretation of the functional association between the middle claw and the serrate accessory setae is supported by the correlation between their positions on the tarsus: on legs I and II the middle claw is directed prolaterally, and most of the setae are on the prolateral side of the tip of the

tarsus; on legs III and IV, the middle claw is directed retrolaterally, and most of the setae are on the retrolateral side of the tip of the tarsus (Nielsen 1931). These asymmetrical positions of the middle claw and the serrated accessory setae are correlated with the positions in which the spider often holds her legs; legs I and II are often directly anteriorly and legs III and IV are directed posteriorly (Nielsen 1931).

Additional evidence favoring this interpretation of a grasping function was obtained when the serrate accessory setae were experimentally removed from the tarsi of all of the legs of *Araneus diadematus* Clerck, 1757 (Araneidae); the spider experienced difficulties in both web construction and in climbing vertical lines (Foelix 1970). Spiders climbed a line five times more slowly after the serrated accessory setae had been removed (Foelix 1970). Comparative morphological data from other species also support these this interpretation. A species which lacks the setal teeth, the amaurobiid *Amaurobius ferox* (Walckenaer, 1830) slipped while attempting to climb a vertical dragline (Nielsen 1931). *Hyptiotes paradoxus* (C.L. Koch, 1834) (Uloboridae) which builds a triangular web, holds a signal line to her web under substantial tension with her legs I for long periods while she waits for prey, both the middle claws and their serrated accessory setae are especially stout and have numerous teeth.

The present paper adds a behavioral dimension to this classic morphological story. The logic is based on the recognition that an orb web spider probably never sees the lines in her web during web construction. Web spiders are known to employ two techniques to find lines to grasp: tapping with their legs to contact lines (like a blind man with his cane) (Vollrath 1992), and "following", in which one leg follows another to grasp a line that the other has already found and grasped (Hingston 1922; Eberhard 1972, 1987a). Following allows more posterior legs to economize on time and effort by following more anterior legs in both space and time. In simple terms, the spider locates and grasps a "new" line with a more anterior "leading" leg as she moves forward,
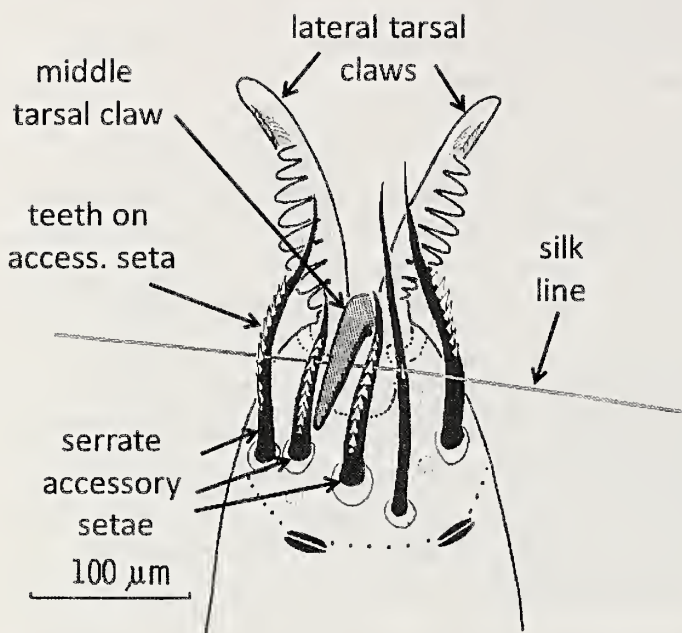
Figure 1.—A schematic representation of how the middle claw of *Araneus diadematus* grasps a line by pressing it against the teeth on nearby serrate accessory setae. The claw is grasping the line only loosely, as it does not cause the line to bend against the setae (from Foelix 2011).

and then moves her more posterior (nearly always the adjacent, ipsilateral) "following" leg forward and grasps this same line; soon afterward, the leading leg usually releases its grip and moves forward. Sometimes a line is passed to two or three more posterior legs in succession.

Following behavior was first described in the sticky spiral construction behavior of *Nephila pilipes* (= *maculata*) (Fabricius, 1793) (Nephilidae) by Hingston (1922), then much later by Eberhard (1972, 1987a) in hub construction behavior of *Uloborus diversus* Marx, 1898 (Uloboridae) and *Leucauge mariana* (Taczanowski, 1881) (Tetragnathidae), and in sticky spiral construction of at least eight genera of Araneidae, three of Tetragnathidae, and one of Nephilidae (Eberhard 1981). In these previous descriptions, all of which were based on observations with the naked eye, the following leg was said to step directly to grasp the line already being grasped by the leading leg. As will be shown below, video recordings have revealed that these descriptions were somewhat imprecise: the following leg usually executes a small "short distance" searching movement, usually lasting on the order of about 0.1s, just before the leg contacts the line and grasps it, rather than moving directly to it.

Short-distance searching movements are extremely predictable in time when a following leg nears the line being held by a leading leg. In addition, the identities and locations of the lines for which the spiders are searching are clear. This makes it possible to study short-distance searching behavior with unusual detail and confidence. There are, for instance, no complications regarding other possible functions of these movements such as locomotion or searches for other objects. It was thus possible to deduce the probable functions of the consistent orientations of legs during short distance searching in relation to tarsal morphology.

The second objective of this paper is to revisit an unresolved problem in understanding how spiders grasp lines. The classic description of grasping assumes that the line is more or less perpendicular to the longitudinal axis of the tarsus (Fig. 1); it does not explain how a line could be grasped when the line is oriented more or less parallel to the long axis of the spider's leg. As noted by Nielsen (1931), spiders nevertheless routinely grasp lines with these orientations. This paper describes observations that suggest how this is done.

METHODS

Video recordings of orb web construction were made with a 30 frames per second (fps) hand-held SONY HDR-SR11 camera equipped with a +2 close-up lens, either in captivity with *Zosis geniculata* (Olivier, 1789) (Uloboridae), *Cyclosa monteverdii* Levi, 1999 (Araneidae), and *Nephila clavipes* (Linnaeus, 1767) (Nephilidae), or in the field (the other species in Table 1). In most species only a single individual was filmed, but in all cases, the web being built was typical and the activity was repeated many times. Not all details were always clear at all times in the recordings, but all characterizations were based on >10 clear cases (see Table 1). Sticky spiral construction in vertical webs was usually filmed while the spider was above as well as below the hub; all descriptions of sticky spiral construction involved spiders in the outer portions of their orbs. Illumination for *Z. geniculata* was a near infra-red light (using the "night shot" feature of the camera); the others were illuminated by ambient light. In descriptions of the construction of spiral lines, the legs on the side farther from the hub are termed "outside" legs (e.g., leg oI), and those nearer the hub are termed "inside" legs (e.g., leg iI); in all cases, none of the lines that were grasped (radii, hub spiral, temporary spiral lines) were sticky.

One leg was characterized as following another if it consistently moved to and grasped a line near the point where that same line was already being held by the leading leg, and if the leading leg soon afterward released its hold on the line (typically it moved forward to find and grasp another line). "Short-distance searching" movements were small amplitude movements (on the order of one or a few diameters of a tarsus) that were executed by the tip of a following leg approximately 0.1s before it grasped the line being held by the leading leg; the movement of the following leg preceding short-distance searching was usually direct, presumably because the approximate location of the line was already known by the spider. Short-distance searching movements differed from the much larger-amplitude "long distance" searching leg movements that were often repeated several times in a row when the spider explored an empty space by waving or tapping with her legs. To improve the clarity of behavioral descriptions, I will employ the illusion (as in other languages such as German, French and Spanish) that all spiders are females; in point of fact, all behavioral observations involved mature females.

Recordings were analyzed frame by frame to determine the side of the leg (prolateral vs. retrolateral) on which a following leg first made contact with the line that it grasped. Particular attention was paid to the movements of following legs in the last frame or two before the leg grasped the line. The direction in which the line was displaced by the following leg in the first image in which contact occurred also gave especially clear

Table 1.—A tentative list of possible uniformities in two details of leg movements, following and prolateral vs retrolateral short-distance searching movements, that were performed during orb construction by a serendipitous sample of species whose construction behavior I happened to have recorded (not all stages are represented for all species). All recordings were made in the field except those of *Cyclosa monteverdii*, *Nephila clavipes*, and *Zosis geniculata*. In most species only a single individual was filmed, but in all cases the activity was repeated many times. Sticky spiral construction was characterized in the outer rather than the inner half of the web, and usually included behavior above as well as below the hub in vertical orbs. One leg was characterized as "following" another if it consistently moved to and grasped a line near to the point where that same line was already held by the other, leading leg, and if the leading leg then quickly released its hold on the line (typically moving forward to find and grasp another line). "Short-distance" searching movements were the generally small amplitude movements executed by the tip of the leg approximately 0.1s before it grasped a line; they were especially clear when one leg was following another (probably because the approximate location of the line was already known by the spider). Legs are indicated by "o" and "i" to indicate their positions during construction: "outer" legs were those directed away from the hub while the spider was spiraling around the web building hub, temporary and sticky spiral lines; "inner" legs were on the side nearest the hub (e.g., Fig. 4A–E). Many of the behavior patterns (both following and short-distance searching) were not absolutely constant, and the characterizations represent the most common types of movements rather than exhaustive lists of all movements. Some species were too small or moved too rapidly (especially *L. mariana*) for me to decipher the movements of certain legs in certain situations; lack of observations is indicated by "–". Inconsistent behavior was also not characterized. Thus, for instance, some interior legs during sticky spiral construction only occasionally followed others, and were not counted as following. In sum, this table does not provide final characterizations of all leg movements, but rather illustrates two apparently general trends in the more consistent and easily observed types of leg movements: legs often follow the immediately anterior ipsilateral leg; and short distance searching movements by legs I and II tended to be prolateral in direction, while those by legs III and IV tended to be retrolateral. Family name abbreviations: AR – Araneidae; NE – Nephilidae; TET – Tetragnathidae; UL – Uloboridae.

Behavioral operation and spider	Following behavior (leading leg – following leg)	*Not* follow any leg	Direction of short-distance searching	
			Prolateral	Retrolateral
A. Secondary radius construction				
Leucauge mariana TET	I-II; II–I; I–I (contralateral)[1,2]	I; II	I; II	–
Micrathena duodecimspinosa AR	I-I[2]	–	–	–
Zosis geniculata UL	oI-iI[2]	–	–	–
B. Hub construction				
L. mariana TET	oI–oII; oII-oIII; oIII–oIV	oI; iIII[3]	–	oIII[4]; oIV[4]
M. duodecimspinosa AR	oI-oII; oII-oIII; oIII-oIV	oI; iIII[3]	oI; oII; iI; iII	oIII; oIV
Z. geniculata UL	oII-oIV; oIV-oIII[5]	oIII; oII	–	–
C. Temporary spiral construction				
L. mariana TET	oII–oI; oIII–oII; oIV-oII[6]	–	–	–
Cyrtophora citricola AR[7]	oI-oII; oII-oIII			–
Nephila clavipes NE	oII–oIII; oIII–oIV; oI-oII[8]	oI; oII; iI; iII; iIII	oI; oII; iI; iII	oIII; oIV
Zosis geniculata UL	oI-oII; oII-oIII; oIV-oII/oIII[9]	iII; iIII[10]	oI?; oII?	oIV
D. Sticky spiral construction				
L. mariana TET	oI–oII; oII–oIII; oIII-oIV?[6]	–	–	oIV(?)
M. duodecimspinosa AR	oII-oIII; oIII-oIV	oII	oI;oII	oIII?; oIV[11]
Gasteracantha cancriformis AR	oI-oII[12]; oII-oIII[13]; oII/oIII-oIV[6]	oII[11]	oI[14]; oII[15]	oIII[14]; oIV
Araneus expletus AR	oII-oIII; oIII–oIV; iI–iII; oI–iI	oII, iI	oI(?); oII(?);iI; iII	oIII; oIV
Cyclosa monteverdii AR	oII-oIII; oIII-oIV	iII; iIII	oII	oIII; oIV; iIII; iIV
N. clavipes NE	oIII-oIV[11]	oIII; oII; oI	–	oIII; oIV
Zosis geniculata UL	oI-oII[16]; oII-oIII[13]; oIII-oII[13] oI-iI[17]; iI-oI[17]; iIII-oIII[18]	–	oI; oII[15]	oIII[15]

[1] all three following sequences were common; some other times these legs did not follow each other

[2] as legs I repeatedly grasped successive possible exit radii; in the *L. mariana* and *M. duodecimspinosa* there was no distinction between "inner" and "outer" leg I

[3] During construction of the first loop the leg did not move at all

[4] The movements were very rapid, so there is some uncertainty in this characterization.

[5] oIV grasped r_n first, then oIII grasped it nearby. But oIV did not then immediately release its grip and move on; instead both legs held the radius as the sticky line was attached between them.

[6] Leg oII often left the radius one or two frames of the video recording before oIV arrived; during this time oIII (which had followed oII) remained holding the same radius. The site grasped by oIV was closer to that grasped by oII than to that grasped by oIII. It is thus not entirely clear whether it should be said that oIV followed either oII or oIII.

[7] construction of non-sticky spiral in dense horizontal sheet

[8] Behavior occurred when spider interrupted temporary spiral construction to lay a tertiary radius

[9] both oII and oIII were on r_n

[10] at least following was not consistent

[11] the tarsus often appeared to slide (probably making contact on its retrolateral side) along r_n before gripping it

[12] oII followed oI to first seize r_n, but later did not follow oI during inner loop localization behavior when it occasionally grasped r_n briefly while it was tapping to locate the inner loop of sticky spiral

[13] hand-over-hand movements reeling in or walking out r_n, with each leg grasping the radius outside the other; often only 1-2 steps.

[14] when reached to grasp r_{n+1}, but not when tap to locate the inner loop

[15] especially clear as oII and oIII pulled in or walked out r_n hand-over-hand

[16] except on the first sticky spiral on r_n, when oI held the temporary spiral and oII grasped r_n.

[17] infrequent

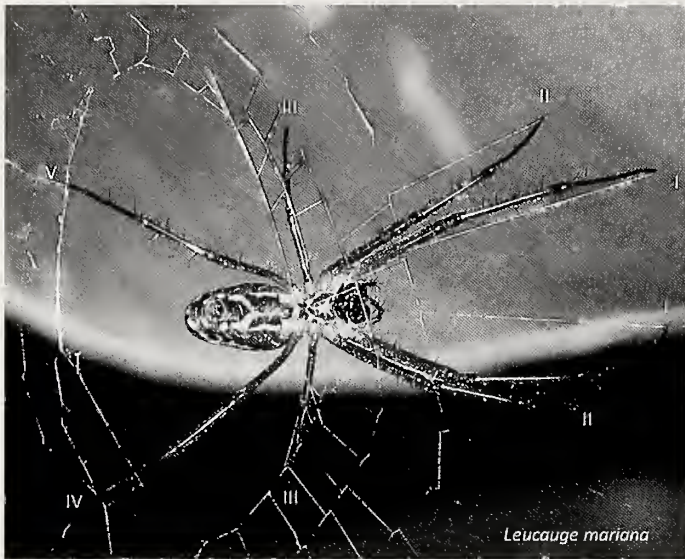[18] only the first step of leg oIII following an attachment

Figure 2.—A mature female *Leucauge mariana* in the typical resting posture at the hub. Legs I and II hold radii in the free zone, each of which is approximately parallel to the long axis of the leg.

Possible ways to grasp lines that are parallel to long axis of the leg (hypotheses):

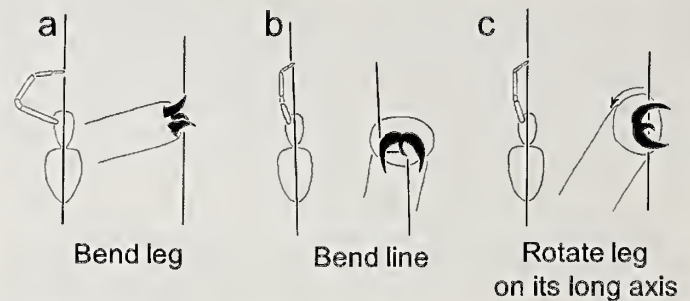Bend leg   Bend line   Rotate leg on its long axis

Figure 3.—Three theoretically possible ways in which a spider might orient her leg to grasp a line with her middle claw that was more or less parallel to the long axis of the leg: (a) bend the leg so that the long axis of the tarsus is perpendicular to the line; (b) bend the line so that it is perpendicular to the long axis of the leg at the point where the middle claw grasps it (Nielsen 1931); and (c) rotate the leg on its long axis so that the middle claw is perpendicular to the line.

indications regarding which side of the leg had made first contact.

The orientation of the tarsal claws with respect to the long axis of the leg while the tarsus grasped a line was checked under a dissecting microscope by observing the tarsi of mature females of the orb-weavers *Leucauge mariana, Argiope argentata* (Fabricius, 1775) (Araneidae), *Z. geniculate, N. clavipes,* and *Kukulcania hibernalis* (Hentz, 1842) (Filistatidae) that were resting immobile at the hub of an orb or other lines (in the case of *K. hibernalis,* in her retreat).

RESULTS

Following behavior.—Following behavior occurred during many stages of construction, including exploration and the construction of the radii, the hub, the temporary spiral, and the sticky spiral (Table 1). It was also taxonomically widespread, and occurred in all of the orb-weavers. Following behavior was sometimes facultatively omitted. For instance, *L. mariana* sometimes altered her usual "explore and then follow" behavior when she was in an area where the lines were so dense that her leg was likely to encounter a line nearby wherever she placed it. Facultative changes also occurred in some other contexts (Table 1). For instance, leg oIII followed leg iIII during sticky spiral construction in *Zosis geniculata* only in making its first step after the spider attached the sticky spiral to a radius.

Short-distance searching behavior.—The following leg often executed a small exploratory movement just before it contacted the line held by the leading leg; these movements probably functioned to locate the line, because the following leg never grasped exactly the same site that was being grasped by the leading leg. The short-distance searching movements made by following legs were asymmetrical in all species (Table 1). Legs I and II consistently moved prolaterally to find and grasp lines; legs III and IV, in contrast, moved retrolaterally. Neither the claws nor the accessory setae were visible in the

video recordings, but the orientations of these movements presumably facilitated grasping the line with the asymmetrically placed middle claws and serrate accessory setae.

Grasping lines nearly parallel to the leg's long axis.—All species that were observed sometimes grasped lines that were more or less parallel to the longitudinal axis of the leg (Fig. 2). Three theoretically possible ways in which a spider might use the classic middle claw grasping mechanism to grasp such lines are illustrated in Fig. 3: bend the leg to orient the tarsus perpendicular to the line; bend the line itself (Nielsen 1931); and rotate the leg on its long axis. Bending the leg was ruled out by the leg positions observed: the long axis of the tarsus of the leg was often more or less parallel to the line (e.g., Fig. 2). Nielsen (1931) thought that, as often occurs with a human's grip on a rope, the line was bent where the tarsus seized it ("... any one pulling at a rope will quite naturally ... [bend] it just at the point where the hand is holding it..." p. 23). Observations under a dissecting microscope of tarsi I of *L. mariana* and *Z. geniculata,* and of tarsi IV of *Argiope argentata* and *N. clavipes* showed, however, that the line was not bent perceptibly in any of these species. Instead, in all four species, the tarsus was rotated on the long axis of the leg, bringing the middle claw into a position that was approximately perpendicular to the line, and thus allowing this claw to grasp the line (Fig. 4). In contrast, the tarsal claws of *K. hibernalis* made widely variable angles with the lines that they grasped, and the portion of the claw that touched the line also varied widely (Fig. 5); rotation, if it occurred, was not dramatic.

DISCUSSION

Following behavior.—The functional significance of following behavior seems clear: by reducing the need for her more posterior legs to wave and explore like a blind man's cane, the spider can move more quickly, and expend less energy. Some spiders, as might be expected, facultatively abandoned following behavior when the lines in the web were dense. The taxonomic distribution of following behavior has not
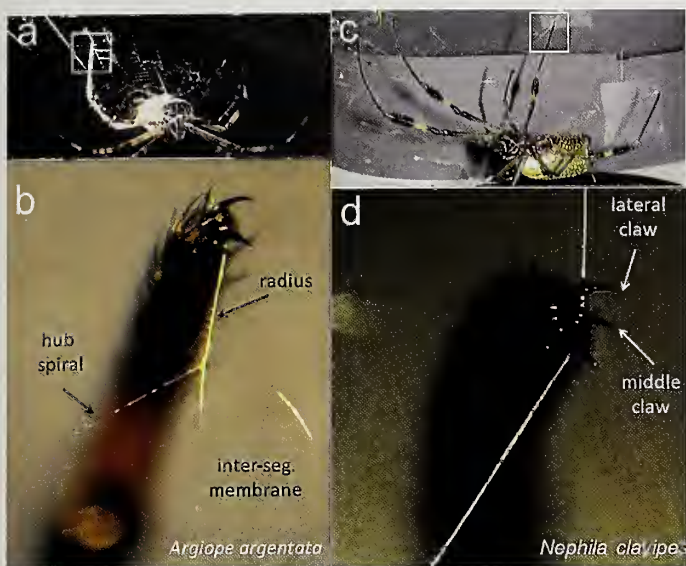
Figure 4.—Positions of *Argiope argentata* (*a*) and *Nephila clavipes* (*c*) as they rested on networks of lines, and closeup images of the tarsal claws of each (*b* and *d* respectively), as indicated by the rectangles in *a* and *c*. In both cases, the claws were rotated substantially on the long axis of the leg, and were approximately perpendicular to the line being grasped. Note the middle claw pressing on the line in *d*. In neither case is the line bent perceptibly to bring it perpendicular to the tarsus.

been determined. It appears to be widely used by orb-weavers (Table 1). To my knowledge, no survey of following in non-orb-weavers has ever been published. Following behavior was apparently absent in early instar nymphs of a ctenizid (likely *Ummidia*) that were filmed as they walked under lines to disperse (W. Eberhard, unpub.). But leg IV of the diplurid *Linothele macrothelifera* Strand, 1908 tended to follow ipsilateral leg III during sheet construction when the spider was walking on leaf litter and on the sheet of silk she had already laid (Eberhard & Hazzi 2013). Following may thus be an ancient trait, and may have already been present when early araneomorph spiders evolved to walk under aerial lines. Similar following behavior has evolved (presumably convergently) in the emesine bug *Stenolemus giraffe* (Hemiptera: Reduviidae: Emesinae), a predator of web spiders; posterior legs follow anterior legs as the bug walks in the webs of its prey (F. Soley, pers. comm.).

Asymmetrical short-distance searching behavior.—The tendency documented here for short-distance searches by legs I and II to be in a prolateral direction, and by legs III and IV in a retrolateral direction, complements the asymmetrical orientation of the middle claws and placement of the serrate accessory setae on these legs, and probably makes it easier for the leg to grasp the line when it makes contact. Presumably the evolution of the asymmetrical tarsal morphology was linked to the advantage of widening the area being searched by making laterally oriented searching movements.

One behavioral exception lends further support to the association between asymmetry in behavior and morphology. Inner loop localization behavior during sticky spiral construc-
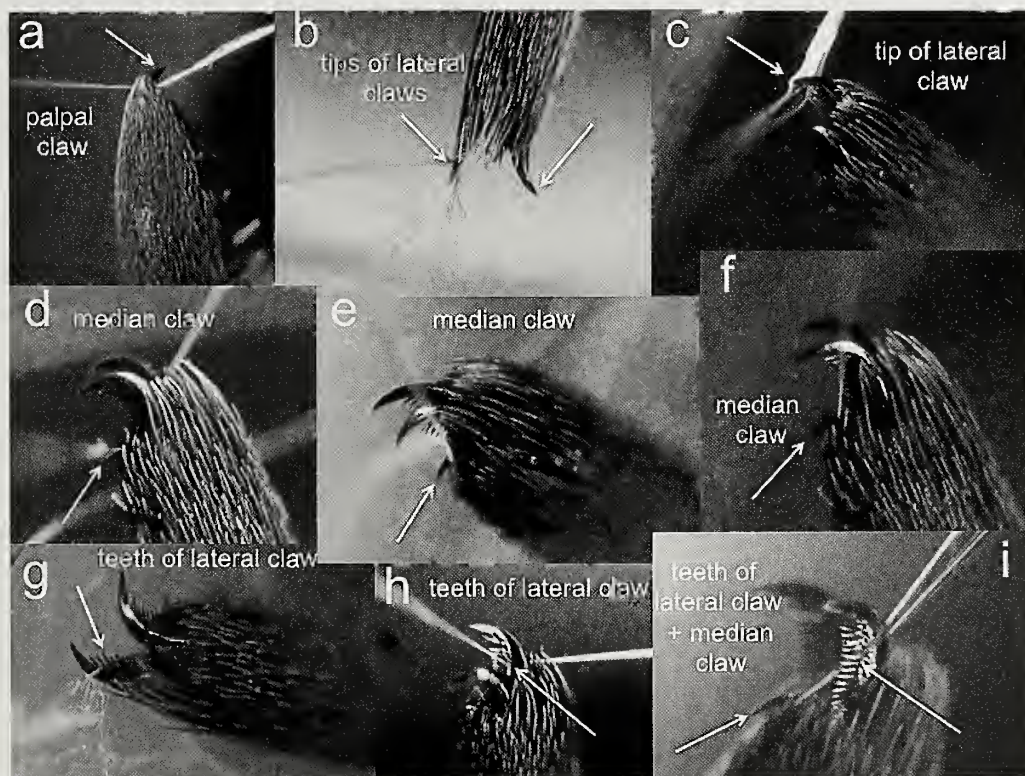

Figure 5.—Variations in the positions of tarsi and the lines that they held while a mature female *Kukulcania hibernalis* rested in her retreat. Lines were snagged by various structures in addition to the middle tarsal claw, and the orientations of lines with respect to the claws varied substantially.

tion involved legs movements to explore to encounter a line, but not to grasp it. Previous, naked eye observations suggested that legs tapped mostly in a dorso-ventral direction in this context (Eberhard 1982). The video recordings in this study confirmed that the movements of leg oI used to contact the inner loop were mainly dorso-ventral rather than lateral in the araneids *Araneus expletus* (O. P.-Cambridge, 1889), *Cyclosa monteverdi*, *Gasteracantha cancriformis* (Linnaeus, 1758), and the uloborid *Zosis geniculata* (leg oI); a dorsally oriented, extension of oIV was employed in the nephilid *Nephila clavipes*. The tapping leg generally touched the inner loop only briefly (usually in only a single frame in the video recordings), and did not seize it.

Rotating legs to grasp lines.—The mechanism by which spiders rotated their legs to grasp lines that were approximately parallel to the leg's long axis is not certain, and will require further work. Two considerations suggest that the claws themselves did not rotate with respect to the tarsus. The three claws are solidly fused, and they have only two tendons attached to them, which are positioned to raise and lower the claws as a unit (Ramirez 2014). Secondly, changing the orientation of the middle claw without changing the positions of the serrate accessory setae against which the middle claw presses silk lines would not be advantageous for grasping lines. This suggests that the apparent rotation observed here occurred at an as yet undetermined more basal articulation. The tibia-patellar articulation is a potential site, as it has only a single condyle and four muscles, and might thus have relatively great mobility (Manton 1977; J. Runge, pers. comm.). Legs are known to rotate somewhat on their long axes when spiders walk upright on the substrate (J. Runge pers. comm.). Further observations will also be needed to determine the taxonomic extent of the ability to rotate legs on their long axes to grasp lines.

The coordination between rotation and other aspects of leg movement as the spider moves in her web also remains to be determined, and may be complex. It seems likely that the spider sometimes first senses the orientation of the line as she is first making contact with it, and then rotates her leg in the process of grasping the line. Such adjustments seem particularly likely to occur during long-distance searching behavior. An observation of *Z. geniculatus* under a dissecting microscope suggested, however, that rotation may sometimes occur earlier. When this spider moved one leg II to grasp a line that was already being held by her other leg II, the moving leg II was already rotated so that its middle claw was approximately perpendicular to this line. During following behavior, the leading leg might provide information on the orientation as well as the location of the line for the following leg. This could enable the spider to adjust the degree of rotation of her following leg before it contacted the line, facilitating its grasp of the line. These are, however, only conjectures at the moment.

Grasping lines in other contexts.—Spiders sometimes snag a line and then slide the tarsus along it smoothly; this is especially clear in species in which the dragline apparently slides through tarsal claw IV while the leg IV is extended posteriorly and is approximately parallel to the drag line. Presumably during sliding the leg is rotated and the middle claw is deflected ventrally only slightly, so that the line is

snagged on its ventral surface, but the claw is not strongly flexed ventrally and the serrated accessory setae are not bent sharply (Fig. 1) (Wilson 1962). I do not know the taxonomic distribution of this use of leg IV. It appears to be widely distributed in Orbicularia; in contrast, some mygalomorph and haplogynes such as filistatids (Eberhard 1986), and the entelegyne web building wolf spider *Aglaoctenus castaneus* (Mello-Leitão, 1942) (Lycosidae) (Eberhard & Hazzi in prep.) were not seen to slide the dragline through any claws (proving the absence of a behavior is difficult, however).

The ability to grasp lines firmly with leg IV probably enables the spider to lay lines under higher tensions than those needed to pull the lines from their spinnerets. Thus, the orb-weaver *Micrathena duodecimspinosa* (O. P.-Cambridge, 1890) (Araneidae) built frame and anchor lines under substantial tension; when she attached an anchor line to the substrate, she held the new line she was producing with one leg IV while she pulled herself forward on the substrate with her more anterior legs just prior to making the attachment (W. Eberhard, unpub.). The spider's movement onto the substrate just preceding an attachment of this sort was accompanied by increased tension on the dragline, as indicated by the angles formed with the web lines to which it was attached and also, in some cases, by displacement of the substrate itself (e.g., bending a flexible leaf). High tensions were particularly clear when the substrate to which the spider was attempting to attach was smooth: she scrabbled with her anterior legs, sometimes for many seconds, before getting a foothold and moving forward slightly and turning to make the attachment. In sum, the spider's ability to grasp her dragline firmly with leg IV allowed her to increase the tension on the line above that needed to pull silk from her spinnerets.

Limitations of this study.—For the orb-weavers of this study, the morphology of the middle claws and the serrated accessory setae, their positions on the tarsi, the following and short-distance searching behavior, rotation of the spider's legs, and the results of previous experimental manipulations of the setae (Foelix 1970), all argue strongly that the middle claws and the associated serrate accessory setae function in grasping silk lines. The distributions of serrate accessory setae and middle claws in other groups suggest, however, that this interpretation is incomplete.

Serrate accessory setae are probably ancient; the accessory setae of the austrochilids in the genera *Thaida* Karsch, 1880 (Ramirez 2014), *Austrochilus* Gertsch & Zapfe, 1955 and *Hickmania* Gertsch, 1958 (Griswold et al. 2005) are similar in form and asymmetry to those of araneids such as *Araneus diadematus*. I have not made a comprehensive review, but perusal of two recent compendia (Griswold et al. 2005; Ramirez 2014) revealed several unexpected combinations of traits. Perhaps the greatest surprise is the senoculid *Senoculus* sp., which has both a middle claw and serrate accessory setae, with both displaced prolaterally on leg I (Ramirez 2014), just like the orb-weavers in the present study; but these are wandering spiders that apparently never make webs (Coville & Griswold 1983) (perhaps the tarsal modifications are used in climbing vertical draglines). Another puzzling combination of traits occurs in the web-building family Psechridae, which have 3 claws and walk under lines in their webs (Bristowe 1930; Eberhard 1987b; Zschokke & Vollrath 1995), but have

claw tufts rather than accessory setae (Griswold et al. 2005). Some spiders have a middle claw but lack serrate accessory setae (Griswold et al. 2005), and hang below lines when they are in their webs: these include Hypochilidae (Shear 1970; Eberhard 1988), Neolanidae (Griswold et al. 2005), Eresidae (e.g., Eberhard 1987b), and Psechridae (Eberhard 1987b; Zschokke & Vollrath 1995). The two compendia also document families in which spiders do not normally hang below their web lines and have tarsi with middle claws but that lack serrate accessory setae, including Amaurobiidae (Bristowe 1958), Oecobiidae (Glatz 1967), Cycloctenidae (Forster & Forster 1973), Desidae (Griswold et al. 2005), Huttonidae (Forster & Forster 1973), Segestriidae (Griswold et al. 2005), and Filistatidae (Griswold et al. 2005) (although a mature female of the filistatid *Kukulcania hibernalis,* which normally walks upright on its web or the substrate over which its web is stretched, chased a prey under a dense sheet of lines in an old web, and then walked hanging from the underside of this sheet with no apparent difficulty).

Nor are the middle claws of web spiders necessarily used to grasp lines. The tarsi of a *K. hibernalis* resting in her retreat illustrated a possible early stage in the evolution of the ability to grasp silk lines: angles between tarsal claws and lines varied widely, as did the portions of the claws which contacted the lines (Fig. 5). In all cases, the lines appeared to be only snagged, rather than grasped. The middle claws of this group may have different functions, such as bracing or hooking against the substrate as the spider moves. Perhaps some serrate setae and middle claws serve to allow the spider to climb vertical lines (though they are certainly not necessary to perform this behavior—see Eberhard 1986). There are still further subtleties in the middle claws of web builders, such as the concave surfaces on the retrolateral sides of the teeth of the middle claw (Nielsen 1931, M. J. Ramírez pers. comm.), whose functional significance is unclear.

In short, there are several mysteries yet to be unraveled regarding how spiders grasp and walk on lines. These remaining mysteries were graphically illustrated by the observation of a young unidentified sparassid, a group which has only two claws and a dense claw tuft rather than serrated accessory setae, literally sprinting up its dragline after being dislodged from its retreat (W. Eberhard, unpub.).

ACKNOWLEDGMENTS

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Functional changes in web design along the ontogeny of two orb-weavers

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Abstract. Orb webs evolved primarily to capture prey, though they also have other functions. Recently, it has been argued that the orb web does not work as a functional unit, but instead some sections or components have presumably been shaped by selection to increase capture success of large prey (relative to the spider size). Changes in these components (e.g., an increase capture area) presumably compromise the design and function of other components (e.g., density of adhesive threads). In this study, we explore the changes in the design of orb webs throughout the ontogeny of two orb-weaving spiders of the genus *Leucauge*: *L. mariana* (Taczanowski, 1881) and *L. argyra* (Walckenaer, 1841). Small nymphs of both species construct webs with a relatively larger capture area and higher density of adhesive spiral loops compared to webs of larger individuals. In addition, small nymphs of *L. argyra* construct webs with more radii. These features probably increase the probability of capturing large prey. Some web features show different trade-offs in the two species. For instance, the number of adhesive threads increases with capture area in webs of *L. mariana*, but decreases in *L. argyra*. The density of adhesive threads in webs of both species decreases as the area of the web increases, but decreases faster in *L. argyra*. Thus, small nymphs are capable of optimizing different structural components of the web to increase the probability of capturing large prey, but the trade-offs between web features vary between species.

Keywords: Web modularity, ontogenetic changes, *Leucauge argyra*, *Leucauge mariana*

Orb-web spiders are sit-and-wait predators whose prey capture success relies on the location and characteristics of the web. The capture success of orb-web spiders is directly related to at least three functions of the web: interception, stopping, and retention of prey (Denny 1976; Eberhard 1986, 1990; Lin et al. 1995; Craig 2003; Herberstein & Tso 2011). Interception of flying, jumping or falling prey depends on the spider's selection of the site to construct its web and on the web size, but stopping and retaining prey without the web breaking is related to the web design and the characteristics of the silk of different threads (Janetos 1986; Barrantes & Triana 2009; Blackledge et al. 2011).

Spiders can increase the probability of intercepting prey by increasing web size (Blackledge & Eliason 2007), but the probability of capturing large, especially profitable prey does not necessarily increase with web size for most species (Eberhard 2014). Combining some hypotheses that explain the function of changes in the density of radii (Zschokke 2002) and variation in the spacing of adhesive spiral turns (Heiling & Herberstein 1998) in different sections of the orb web, Eberhard (2014) proposed a "multitrap functional approach" to explain the function of the different sections of an orb web. This approach assumes that the probability of intercepting prey is correlated with web size, but the probability of stopping larger, especially profitable prey increases with radius density, and the probability of retaining this type of prey increases with the density of adhesive spiral threads.

Capturing large, profitable prey is uncommon for orb-web spiders, but their growth and reproduction rely heavily on such prey (Venner & Casas 2005). Thus, orb webs are likely designed for these rare, but profitable events, without abandoning the capture of small prey. Small prey presumably serve to sustain spiders until the next large catch (Venner & Casas 2005). Furthermore, energy requirement varies across spiders' sizes and it is known that small spider species (and presumably small young spiders, Mayntz et al. 2009) have a higher metabolic rate

(Anderson 1970, 1996; Humphreys 1977). This sets a different trade-off for small spiders, which are expected to require a proportionally larger amount of energy than large spiders, but are likely to have fewer resources to allocate to web construction (e.g., body fat and proteins).

The multitrap functional approach allows for specific predictions of changes in web design according to the spiders' energy requirements. Thus, if small spiders have a higher metabolic rate and require a relatively larger amount of energy for successful development and growth than large spiders, more large, profitable prey (relative to the spider body size) are required (the rare, large prey hypothesis; Blackledge et al. 2011). Additionally, if webs of small spiders are targeting rare large prey, these webs are expected to be larger, to have a higher density of radii to stop and dissipate the kinetic energy of a moving prey, and to have higher density of adhesive threads (i.e., more closely spaced adhesive spiral loops along radii) to improve retention of large prey (Eberhard 1990; Blackledge et al. 2011; Sensenig et al. 2012; Eberhard 2014). Some of these conditions are thought to be mutually exclusive, e.g., increasing density of adhesive spiral threads could result in smaller orbs, potentially decreasing insect interception, because spiders have a finite amount of silk (Blackledge & Zevenbergen 2006; Blackledge & Eliason 2007).

There are at least three factors – spider size, the relative sizes of its silk glands, and food supply (which is expected to affect silk production) (Mayntz et al. 2009) – that might affect web size and density of threads (e.g., adhesive threads and radii) of orb webs. We examine here the correlative effect of spider size and body condition (measured as residuals from the regression of maximum cephalothorax width vs. maximum abdomen width, Jakob et al. 1996) on capture area, number of loops of adhesive threads, density of adhesive capture threads, and number of radii in two species of the genus *Leucauge*: *L. argyra* (Walckenaer, 1841) and *L. mariana* (Taczanowski, 1881) (Tetragnathidae). Specifically, we predict that (a) small
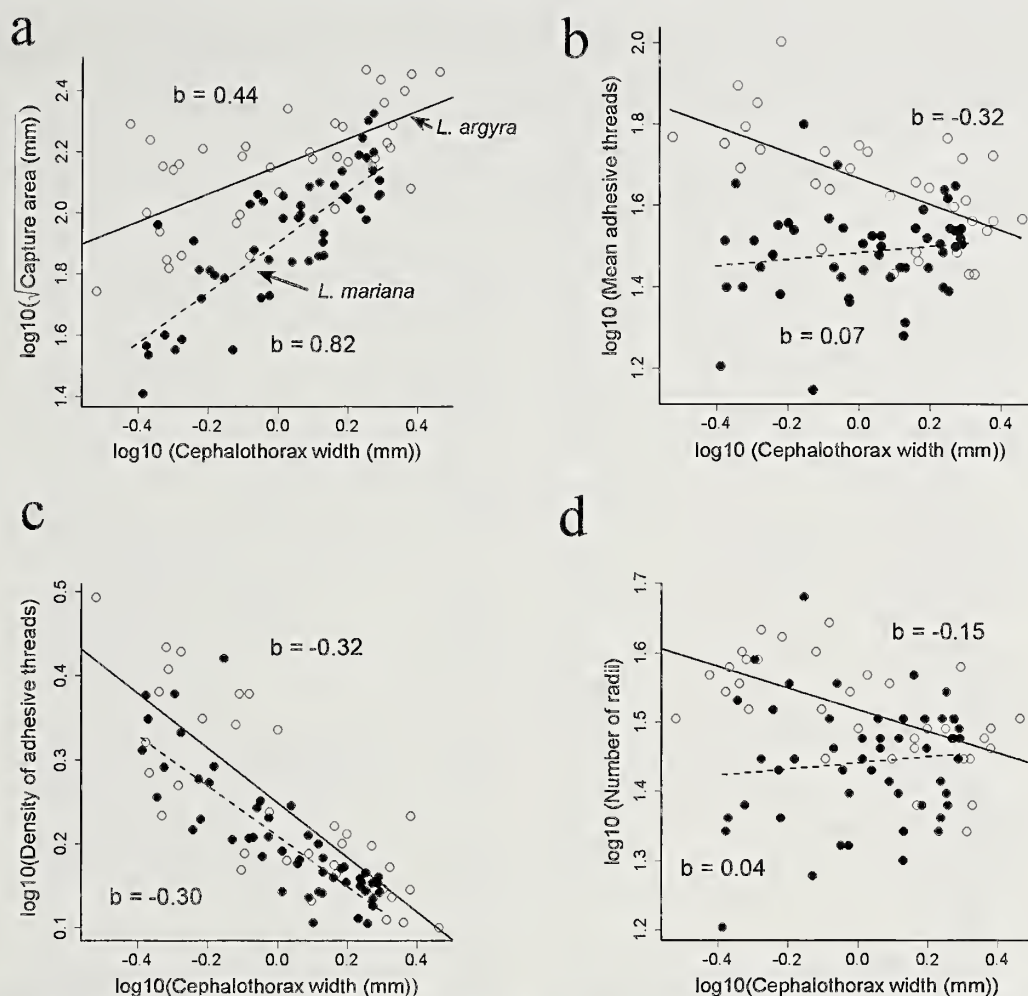
Figure 1.—Relationships between body size (cephalothorax width) and four response variables. (a) Capture area increases with size in both species. (b) The mean number of adhesive threads (sticky spiral loops) decreases with body size in *L. argyra* but not in *L. mariana*. (c) Density of adhesive threads (no. sticky spiral loops/ radii length along capture area) decreases with body size in both species. (d) Number of radii decreases with body size in *L. argyra* but not in *L. mariana*. The value of the slope, b, for each species is shown within each figure.

spiders should construct proportionally larger webs with higher density of adhesive capture threads and higher density of radii, to increase the probability of capturing large, profitable prey to meet their higher energy requirements; (b) spiders with poor body condition would construct larger webs independent of their body size, assuming that interception and prey capture increase with area of the orb web. The first prediction would be validated if small spiders construct proportionally larger and denser webs than large spiders (i.e., negative allometries log-log linear models), and the second prediction would be validated if large or small poorly fed spiders produce larger webs than well-fed spiders of the same size.

METHODS

Focal species.—Both *Leucauge* species construct their orb webs on early second growth vegetation, along forest edges between 0.20 to 1.70 m above ground (Eberhard & Huber 1998; Aisenberg & Barrantes 2011). Most webs vary from nearly horizontal to ca. 20° (GB, unpublished data) and webs of juveniles have a tangle above and below the orb (adults

occasionally have also tangles; Triana et al. 2011); these tangles are denser in *L. argyra*. In Costa Rica, *L. argyra* occurs from sea level to approximately 500 m and *L. mariana* primarily from 700 to 1800 m.

General conditions.—We photographed webs and collected spiders: *L. argyra* at El Silencio, Quepos, Puntarenas province (09° 24′ N, 84° 01′ W, elevation 46 m above sea level.) in January 2013, and *L. mariana* on the campus of the University of Costa Rica (9° 54′ N, 84 °03′ W; elevation 1200 m), San José Province, Costa Rica, and Parque del Este, La Unión, Cartago, Costa Rica (9° 56′ N, 84° 00′ W; elevation 1400 m). We coated each web with talcum powder; placed a 1 cm long piece of graph paper on or just beside the web to serve as a scale prior to taking each picture; and then preserved the spider in 80% ethanol. All webs were photographed early in the morning (0700-1000) to include only the first of several webs these spiders typically construct each day. We found only five webs (3 of *L. mariana*, 2 of *L. argyra*) with a clear indication of spiders' prey capture (some spiral turns collapsed and the spider feeding on prey), and we had no control for the recent feeding history of spiders.

Table 1.—Number of estimated parameters [K], and results of Akaike information criterion [AIC], Δ_i(AIC) [AICi – min(AIC)], Akaike weights [w_i(AIC)] and Bayesian information Criterion [BIC] for the different models constructed for each response variable. ■ Indicates the optimal model for each response variable. Ceph = cephalothorax width.

Model rank	Explanatory variable	K	AIC	Δ_i(AIC)	w_i(AIC)	BIC
	Leucauge argyra					
Response variable: Capture area						
	ceph * resid	4	-21.41	3.68	0.12	-13.93
	ceph + resid	3	-21.31	3.78	0.11	-15.20
	■ceph	2	-25.09	0	0.76	-20.42
Response variable: Mean no. spiral loops						
	ceph * resid	4	-35.46	5.15	0.07	-28.29
	ceph + resid	3	-35.40	5.21	0.06	-30.13
	■ceph	2	-40.61	0	0.87	-36.12
Response variable: No. radii						
	ceph * resid	4	-71.72	7.74	0.02	-64.71
	ceph + resid	3	-73.57	5.89	0.05	-67.83
	■ceph	2	-79.46	0	0.93	-75.07
Response variable: Density of adhesive spiral loops						
	ceph * resid	4	-20.00	3.32	0.14	-12.83
	ceph + resid	3	-19.25	4.07	0.10	-13.39
	■ceph	2	-23.32	0	0.76	-18.83
	Leucauge mariana					
Response variable: Capture area						
	ceph * resid	4	-57.70	0	0.53	-48.35
	ceph + resid	3	-53.11	4.59	0.05	-45.54
	■ceph	2	-57.23	0.47	0.42	-51.50
Response variable: Mean no. spiral loops						
	ceph * resid	4	-60.52	0	0.56	-51.16
	ceph + resid	3	-56.58	3.94	0.08	-49.01
	■ceph	2	-59.69	0.83	0.37	-53.95
Response variable: No. radii						
	ceph * resid	4	-80.80	4.97	0.05	-71.65
	ceph + resid	3	-81.01	4.76	0.05	-73.61
	■ceph	2	-85.77	0	0.57	-80.15
Response variable: Density of adhesive spiral loops						
	ceph * resid	4	-79.42	3.65	0.12	-70.27
	ceph + resid	3	-79.09	3.98	0.10	-71.68
	■ceph	2	-83.07	0	0.77	-77.45
	Both species					
Response variable: Capture area						
	spp * ceph * resid	8	-79.25	3.88	0.11	-57.70
	spp * ceph+ resid	5	-78.24	4.89	0.07	-63.66
	spp + ceph + resid	4	-70.48	12.65	0.00	-58.26
	■spp * ceph	4	-83.13	0	0.80	-70.92
	spp + ceph	3	-75.27	7.86	0.02	-65.46
	spp	2	-21.07	62.06	0.00	-13.67
	ceph	2	-38.53	44.6	0.00	-31.13
Response variable: Mean no. spiral loops						
	spp * ceph * resid	8	-97.98	4.13	0.10	-76.66
	spp * ceph+ resid	5	-97.75	4.36	0.09	-83.31
	spp + ceph + resid	4	-88.53	13.58	0.00	-76.43
	■spp * ceph	4	-102.11	0	0.79	-90.02
	spp + ceph	3	-93.02	9.09	0.01	-83.30
	spp	2	-92.29	9.82	0.01	-84.96
	ceph	2	-59.77	42.34	0.00	-52.44
Response variable: No. radii						
	spp * ceph * resid	8	-149.49	12.82	0.00	-128.52
	spp * ceph+ resid	5	-155.62	6.69	0.02	-141.40
	spp + ceph + resid	4	-152.96	9.35	0.00	81.48

Table 1.—Continued.

Model rank	Explanatory variable	K	AIC	Δ_i(AIC)	w_i(AIC)	BIC
	■spp * ceph	4	-161.54	0.77	0.36	-149.62
	spp + ceph	3	-158.81	3.50	0.09	-149.24
	spp	2	-162.31	0	0.53	-155.09
	ceph	2	-149.96	12.35	0.00	-142.74
Response variable: Density of adhesive spiral loops						
	spp * ceph * resid	8	-92.82	11.59	0.00	-71.73
	spp * ceph+ resid	5	-94.66	9.75	0.01	-80.37
	spp + ceph + resid	4	-99.29	5.12	0.06	-87.32
	spp * ceph	4	-99.76	4.65	0.08	-87.80
	■spp + ceph	3	-104.41	0	0.84	-94.78
	spp	2	-7.38	97.03	0.00	-0.13
	ceph	2	-87.11	17.3	0.00	-79.86

Trade-off between variables

Response variable: Capture area						
	spp * ceph * radii	8	-92.51	1.20	0.29	-71.53
	spp * radii + ceph	5	-91.68	2.03	0.19	-77.46
	■spp + ceph + radii	4	-93.71	0	0.52	-81.80
Response variable: Mean no. spiral loops						
	spp * ceph * capt	8	-122.91	7.97	0.02	70.45
	■spp * capt + ceph	5	-130.88	0	0.85	71.44
	spp * ceph + capt	5	-128.06	2.82	0.21	70.03
	spp + ceph + capt	4	-127.21	3.67	0.16	68.61
Response variable: Density of adhesive spiral loops						
	spp * ceph * capt	8	-124.53	8.34	0.01	71.27
	spp * ceph + capt	5	-125.24	7.63	0.02	68.62
	■spp * capt + ceph	5	-132.87	0	0.95	72.44
	spp + capt + ceph	4	-126.48	6.39	0.04	68.24

On each web photographed, we measured the capture area, the length of the longest radius along the capture area from the inner-most spiral turn (external edge of the free zone) to the outermost adhesive spiral turn, and along the radius opposite to the longest radius; we also measured the distance between sticky spiral threads along both of these radii, following Barrantes & Eberhard (2012). We used ImageJ (Rasband 2016) to measure all features on each web. Additionally, we calculated the density of adhesive spiral loops: the number of adhesive spiral loops/radius length along the capture area for the longest radii and those opposite the longest. For each spider, we measured the greatest eephalothorax and abdomen width using Dino-Eye Eyepiece digital color camera (Model AM423X) attached to the ocular of a Wild Model M3Z dissecting microscope (Wild Company, New York, USA). We used the residuals from the regression of maximum cephalothorax width vs. maximum abdomen width as a proxy of body condition (Jakob et al. 1996). We ineluded a wide range of spider sizes that probably covered from the first stage out of the egg sae to adults (this based on sizes and some differences in eoloration seen in spiderlings of these species); size of spiders correlates with spiders' developmental stages (Barrantes & Madrigal-Brenes 2008; Foelix 2011). We deposited voucher specimens in the Museo de Zoología, Escuela de Biología, Universidad de Costa Rica.

Statistical analyses.—We used Generalized Least Squares Models (GLS; library nlme, using restricted maximum likelihood method-REML) to test the effect of spider size (i.e., cephalothorax width) and body condition on the square root of the capture area, mean number of adhesive spiral loops along

the longest and the opposite radii, density of adhesive spiral threads, and number of radii. We ran the analyses first for each species alone, and then included both species to compare them. We also evaluated if species trade off differently for different web features. Specifically, we first tested the effect of number of radii and spider size on capture area and mean number and density of adhesive spiral loops, and then tested the effect of capture area and spider size on mean number and density of adhesive spiral threads between both species. In the first case, we selected number of radii as the predictor variable because spiders construct all radii first, prior to laying the adhesive threads, and in the second case, the capture area is defined by where the spider places the first turn of the adhesive thread, so that the number of turns of the adhesive spiral is likely a subsequent decision the spider makes after deciding where to place the first turn (Barrantes & Eberhard 2012). In cases when AIC values in models with interaction term (spp * predictor variable) were similar to the AIC of the optimal model (AIC differ by two or fewer units), we favored the first. This allowed us to compare the trade-offs of web features between both species. When the selected models included the interaetion term, spider spp*cephalothorax, the interaction term tested whether body size (cephalothorax width) of both species scale similarly (not significant) or not (significant) for each of the response variables.

We performed a series of models for each response variable and selected the model that gives the most aecurate description of the data based on AIC (Akaike Information Criterion) and AIC weights (Wagenmakers & Farrell 2004) (Table 1). We

Table 2.—Effect of spider size (cephalothorax width = ceph.) on five response variables: capture area (capt. a), mean number of sticky spiral loops, density (mean number of sticky spiral loops/mean radii length), and number of radii for *Leucauge argyra* (A), *L. mariana* (B), and between both species (C) based on Generalized Least Squares Models. It also includes the trade-offs between web features for both species (D). All numerical variables were log$_{10}$ transformed.

A — *Leucauge argyra*

Response variable: Capture area

Effect	Coefficient	SE	T	P
intercept	2.16	0.02	90.28	<0.0001
ceph.	0.39	0.09	4.57	0.0001

Response variable: Mean no. spiral loops

intercept	1.67	0.02	88.01	<0.0001
ceph.	-0.34	0.07	-4.88	<0.0001

Response variable: No. radii

intercept	1.52	0.01	147.69	<0.0001
ceph.	-0.16	0.04	-4.44	0.0001

Response variable: Density

intercept	-0.15	0.02	-6.21	<0.0001
ceph.	-0.75	0.09	-8.26	<0.0001

B — *Leucauge mariana*

Response variable: Capture area

intercept	1.91	0.02	111.80	<0.0001
ceph.	0.82	0.08	9.96	<0.0001

Response variable: Mean no. spiral loops

intercept	1.48	0.02	88.49	<0.0001
ceph.	0.07	0.08	0.86	0.3909

Response variable: No. radii

intercept	1.44	0.01	114.56	<0.0001
ceph.	0.04	0.06	0.74	0.4632

Response variable: Density

intercept	-0.22	0.01	-17.26	<0.0001
ceph.	-0.75	0.06	-12.29	<0.0001

C — Both species

Response variable: Capture area

Effect	Coefficient	SE	T	P
intercept	2.16	0.02	99.02	<0.0001
L. mariana	-0.25	0.03	-8.60	<0.0001
ceph.	0.39	0.08	5.01	<0.0001
spp * ceph.	0.43	0.12	3.61	<0.0001

Response variable: Mean number of sticky spiral loops

Effect	Coefficient	SE	T	P
Intercept	1.67	0.02	84.28	<0.0001
L. mariana	-0.20	0.03	-7.70	<0.0001
ceph.	-0.34	0.07	-4.67	<0.0001
spp * ceph.	0.41	0.11	3.84	<0.0001

Response variable: Number of radii

Effect	Coefficient	SE	T	P
intercept	1.52	0.01	112.11	<0.0001
L. mariana	-0.08	0.02	-4.51	<0.0001
ceph.	-0.16	0.05	-3.37	0.0012
spp * ceph.	0.21	0.07	2.91	0.0047

Response variable: Density of adhesive spiral loops

Effect	Coefficient	SE	T	P
intercept	-0.15	0.02	-7.84	<0.0001
L. mariana	-0.07	0.02	-2.74	0.0074
ceph.	-0.75	0.05	-14.21	<0.0001

D — Trade-off between variables

Response variable: Capture area

Effect	Coefficient	SE	T	P
intercept	1.24	0.48	2.58	0.0116
L. mariana	-0.32	0.56	-0.57	0.5667
ceph.	0.69	0.06	11.04	<0.0001

Table 2.—Continued.

radii	0.59	0.31	1.86	0.0666
spp * radii	0.11	0.38	0.29	0.7697

Response variable: Mean number of adhesive threads

Effect	Coefficient	SE	T	P
intercept	0.055	0.18	0.30	0.7632
L. mariana	-0.09	0.02	-4.40	<0.0001
ceph.	-0.06	0.04	-1.34	0.1831
radii	1.06	0.12	8.88	<0.0001

Response variable: Mean number of adhesive threads

Effect	Coefficient	SE	T	P
intercept	0.95	0.23	3.98	0.0001
L. mariana	-0.73	0.23	-3.16	0.0022
ceph.	-0.48	0.07	-7.35	<0.0001
capt. a	0.33	0.11	3.03	0.0032
spp * capt. a	0.32	0.11	2.93	0.0044

Response variable: Density

Effect	Coefficient	SE	T	P
intercept	1.38	0.23	5.97	<0.0001
L. mariana	-0.93	0.22	-4.15	<0.0001
ceph.	-0.44	0.06	-6.90	<0.0001
capt. a	-0.71	0.11	-6.66	<0.0001
spp * capt. a	0.36	0.11	3.41	0.0010

log$_{10}$ transformed variables, and, in all cases, these transformed variables fit well the assumptions of normality of residuals and homogeneity of variances. In addition, we regressed body size against each of the response variables to obtain each species' slope to show graphically the effect of the interaction term. We used the R statistical language, version 3.0.1 (R Development Core Team 2014) for all analyses.

RESULTS

We analyzed four features of the orb web of *L. argyra* and *L. mariana* which are probably directly related to prey capture success: capture area, mean number of adhesive spiral loops, number of radii, and density of adhesive spiral loops.

Intra-specific analyses.—In *L. argyra*, small nymphs constructed webs with larger capture area, higher density of adhesive spiral loops, larger mean number of adhesive spiral loops, and more radii than larger individuals (slopes significantly lower than 1 in a log-log scale) (Table 2A, Fig. 1).

In *L. mariana*, small individuals constructed webs with proportionally larger capture area and higher density of adhesive spiral loops, but the size of spider did not have a significant effect on the mean number of adhesive spiral loops or the number of radii (Table 2B, Fig. 1). The selected models, based on AIC and other additional parameters, included only size (cephalothorax width) as the predictor variable (Table 1). In addition, in the excluded models, neither the body condition (Fig. 2) nor any interaction had a significant effect on the response variables.

Inter-specific analyses.—All four web features were significantly greater in webs of *L. argyra* than in webs of *L. mariana* (Table 2C). Small individuals of *L. argyra* constructed webs with greater capture area than did individuals of equal size of *L. mariana* (Fig. 1a; Table 2C). For both species, the capture area of the web increased with the spider size, but capture area increased at a greater rate in *L. mariana* (b = 0.82) than in *L.*
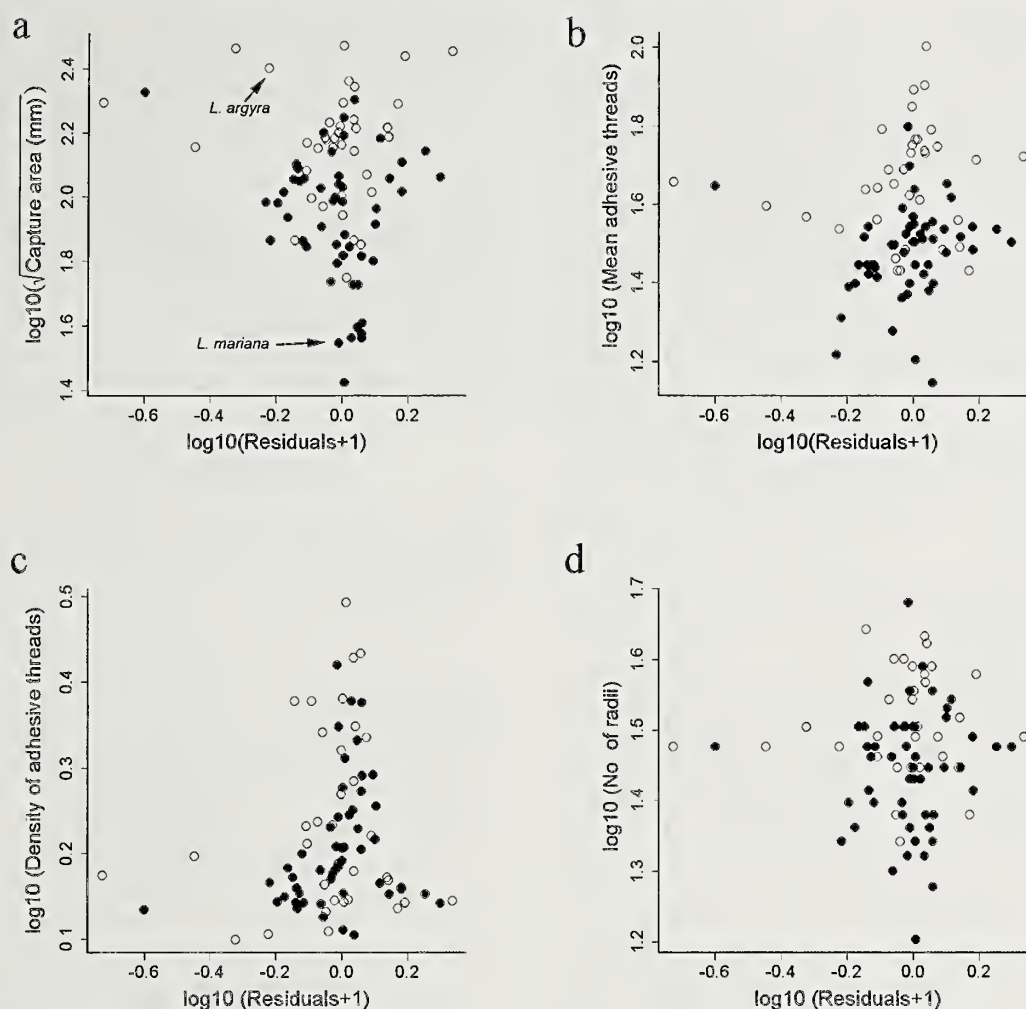
Figure 2.—Relationships between spider size and body condition (measured as residuals from the regression of maximum cephalothorax width vs. maximum abdomen width; Jakob et al. 1996) and four response variables for *L. argyra* and *L. mariana*. (a) Capture area. (b) Mean number of adhesive threads (sticky spiral loops). (c) Density of adhesive threads (no. sticky spiral loops/ radii length along capture area). (d) Number of radii. Residuals did not have a significant relationship with any of the response variables.

argyra (0.44), which resulted in a significant interaction between slopes of both species (Table 2C; Fig. 1a).

The number of adhesive spiral loops decreased drastically as the size of *L. argyra* spiders increased, but the size of the spider had little effect on the number of adhesive spiral loops in webs of *L. mariana* (Table 2C; Fig. 1b). Small *L. argyra* constructed webs with more adhesive spiral loops than large spiders, but the size of *L. mariana* spiders was not correlated with the number of adhesive spiral loops (Table 2C, Fig. 1b). The density of adhesive spiral loops decreased significantly with spider size in both species (Table 2C; Fig. 1c).

Small nymphs of *L. argyra* constructed webs with more radii than did small nymphs of *L. mariana* (Fig. 1d, Table 2C), however, the number of radii decreased rapidly with body size in *L. argyra*, but not in *L. mariana* (Fig. 1d). The body condition of spiders did not correlate with variation of any of the response variables.

Trade-offs between web features.—The trade-off between pairs of web features varied among features and between both species (Table 2D, Fig. 3). The number of adhesive threads increased with the number of radii in webs of both species

(Table 2D, Fig. 3b), and increased with capture area in webs of *L. mariana*, but decreased in webs of *L. argyra* (Fig. 3c). Similarly, the capture area increased in webs of *L. mariana*, but decreased in webs of *L. argyra* with the number of radii (Fig. 3a). The density decreased with capture area in both species, but did so faster in *L. argyra* (Fig. 3d).

DISCUSSION

In general, our results support the hypothesis that the higher energy requirement of small spiders could influence those web features directly related with increasing capture of large prey, but the two species differed in several respects. The large number of radii in webs of small *L. argyra* is expected to increase the probability of stopping large prey as predicted for the "the radius density hypothesis" (Zschokke 2002; Eberhard 2014); more radii impacted by a prey will more effectively absorb the prey's momentum. The capture area was also greater for webs of *L. argyra* than for *L. mariana* at nearly all spider sizes (Fig. 1), but capture area increased faster relative to the spider size in *L. mariana* (Fig. 1). We do not have information that explains the differences in this relationship,
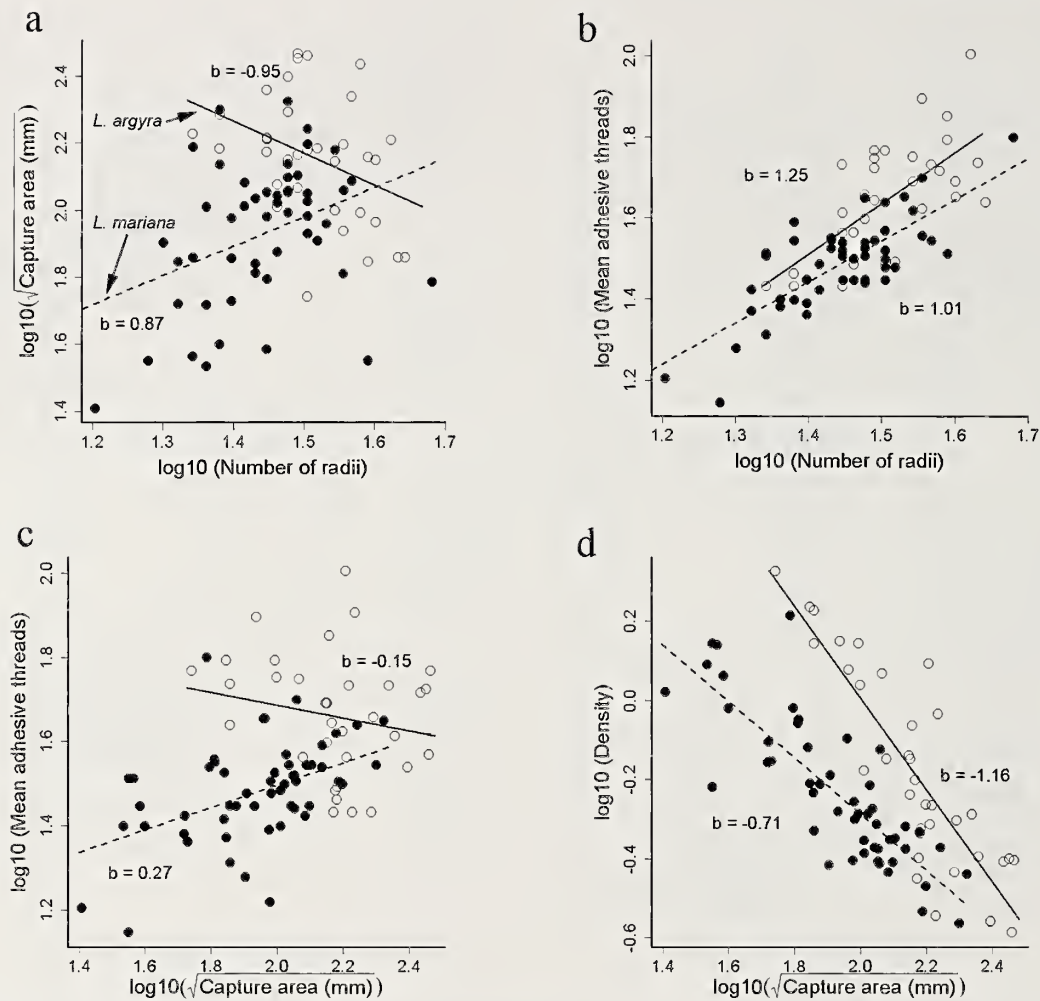
Figure 3.—Relationships between web features. (a) Capture area increases in *L. mariana* but decreases in *L. argyra* with the number of radii. (b) The mean number of adhesive threads increases with the number of radii in both species. (c) The mean number of adhesive threads increases in *L. mariana* but decreases in *L. argyra* as the capture area increases. (d) The density of adhesive threads decreases with the capture area in both species. The value of the slope, b, for each species is shown within each figure.

particularly considering that the ecology of both species is similar in several respects: their microhabitats have similar structure (e.g., early second growth), and their prey overlap at least at family level (Hodkinson 2005, GB unpubl. data). However, different stages in each species could still select particular insect sizes (based on their web design), for which data is unavailable.

There is a gradual change in the design of the web of both species in terms of capture area, density of adhesive threads, and number of radii (in *L. argyra*) as the spider grows. Radii are essential for stopping, particularly large prey. These are structural threads that effectively dissipate the kinetic energy of the flying, or falling prey. Consequently, the probability of capturing proportionally large prey increases in small spiders with the number of radii that a prey impact. A higher density of adhesive threads presumably increases prey retention and consequently the capture success, again in small spiders. This occurs because a higher number of adhesive threads adhere to the prey, retaining it long enough for the spider to deliver the attack (Eberhard 1986, 1990; Lin et al. 1995; Craig 2003; Blackledge et al. 2011; Herberstein & Tso 2011). Web features suggest that glands to produce adhesive threads (i.e.,

aggregate, ampullate, and flagelliform glands) may have different rates of silk production in small nymphs of both *Leucauge* species, which allow them to increase both area and density of adhesive threads. The change in density of adhesive spiral loops in webs of different size could also be influenced by other factors: the spider's morphology, metabolic rate, body condition, remaining amount of silk in glands, prey type and prey abundance (Eberhard 1988, 1990; Blackledge 2012), and wind condition. For instance, in webs of small nymphs, the higher density of radii could also maintain the thinner adhesive threads in place, preventing them from sagging and sticking together under windy conditions (Sensenig et al. 2010; Eberhard 2014). Another alternative explanation is that small nymphs add more threads to their webs to compensate for their proportionally thinner threads, as it occurs in small spider species (Sensenig et al. 2010).

Despite the general pattern found in webs of both spiders, some web features trade off differently between both species. Notably, the number of adhesive threads increases in *L. mariana*, but decreases in *L. argyra* as the capture area increases, and the density of adhesive threads decrease with capture area in both species, but faster in *L. argyra*. This

suggests that there may be species specific trade-offs between web features to increase capture success of large prey, but until the trade-offs between web features of more species are known, this hypothesis will remain open.

The body condition did not correlate with variation of web features. For the body condition to affect web features, the spiders should be under poor feeding conditions for long periods of time (Sherman 1994; Heiling & Herberstein. 2000). However, it is unlikely that for generalist spiders like *Leucauge*, prey availability decreases so drastically in non-seasonal rain forests as to affect silk production and web features. At least for some orb web spiders, prey capture did not decrease during the driest periods in a relatively seasonal rain forests (Robinson & Robinson 1973).

ACKNOWLEDGMENTS

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Orb web architecture of *Wixia abdominalis* O. Pickard-Cambridge, 1882 (Araneae: Araneidae): intra-orb variation of web components

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Abstract. The distribution of sticky spirals and radii within orb webs is usually not uniform. Distinct patterns of silk investment in inner and outer portions of the orb may influence the web's capacity to stop and retain specific prey types. Several incidental and functional hypotheses have been proposed previously to explain the variation in web patterns. Herein, we describe the webs built by spiders of the monospecific genus *Wixia* O. Pickard-Cambridge, 1882 (Araneidae) and evaluate web-building hypotheses, considering the presence of a free-sector, vertical symmetry, sticky spiral distribution and radii spacing. Because all information available on the ecology of *Wixia* is restricted to the species that were subsequently transferred to other araneid genera, there is no information about the webs of the last species remaining in this genus, *Wixia abdominalis* O. Pickard-Cambridge, 1882. We observed that this spider builds complete orbs, but some individuals add a free-sector, remaining resting on a twig above the orb and holding a signaling thread. On the upper part of the orb the spiral distribution follows the pattern of increasing densities from the edge to the hub. However, on the lower part of orbs, this pattern is seen only in complete webs; in contrast, in webs with a free-sector the pattern of spiral distribution observed in lower part of webs is homogeneous from the edge to the hub. We discuss possible implications of the web structure of *W. abdominalis* for prey capture and how the incidental and functional hypotheses may explain the patterns of spiral spacing observed in this species.

Keywords: Free-sectors, sticky spiral spacing, prey capture, web-building hypotheses

Webs spun by araneoid ecribellate orbicularians are composed of distinct types of silk, each one with particular mechanical properties and functions (Blackledge et al. 2011). The non-adhesive radii, for example, are responsible for stopping prey by absorbing its kinetic energy upon impact with the web (Sensenig et al. 2012). Sticky spirals, on the other hand, retain prey in the web long enough to the spider to move to the interception site and subdue the prey (Blackledge & Zevenbergen 2006). In addition, spiders may regulate vertical displacement of the hub from the web's geometric center to increase the probability of prey capture (Nakata & Zschokke 2010; Zschokke & Nakata 2010; Blackledge et al. 2011). Therefore, measurements of the spacing of sticky spirals and radii, and vertical asymmetry of the web provide important information regarding the spider's foraging behavior.

Despite their apparent geometrical uniformity, there is significant inter- and intraspecific variation in orb web design (e.g., presence and types of shelters; free sectors; patterns of symmetry; presence, shape, and composition of stabilimenta) (Manicom et al. 2008; Blackledge et al. 2011; Gonzaga & Vasconcellos-Neto 2012; Eberhard 2014). For example, sticky spiral distribution is usually not uniform from the edge to the hub within orb webs (Herberstein & Heiling 1999; Zschokke 2002; Eberhard 2014). Moreover, the distribution of sticky spirals typically varies between the upper and lower parts of the web, as does distribution of radii (Zschokke & Nakata 2015). These features are, in some way, linked with web dimensions. For example, the more extensive the web part (upper or lower) is, the greater the spacing between the spirals and the more parallel the radii will be (Zschokke & Nakata 2015). However, the upper part typically has a lower density of sticky spirals, and the radii are less parallel than are those in the lower part. This difference can persist even in symmetric webs (Zschokke & Nakata 2015).

Several incidental and functional hypotheses have been proposed to explain intra-orb variation in sticky spiral and radii distribution (see Eberhard 2014), as well as differences in investment between the upper and lower parts of the web. Incidental hypotheses are explanations associated with physical or physiological constraints during web building (Eberhard 2014). The first incidental hypothesis, hereafter "Peters' segment rule," states that the gradual decrease in sticky spiral spacing from the edge to the hub is a consequence of the geometric spacing between adjacent radii, which decreases in the same direction. Therefore, spiders would adjust sticky spiral spacing based on the distance between adjacent radii (Peters 1954). According to Peters' segment rule, it is expected that sticky spiral spacing always increases from the hub to the edge, and this increase should be greatest where adjacent radii are less parallel. The second incidental hypothesis is referred to as the "energetic constraint hypothesis" (Herberstein & Heiling 1999). Because spiders usually have to lift their abdomens to make attachments above the hub, the greater spiral spacing in the upper part of the web is a result of the larger energetic cost imposed during the web building in that part. As the spider fixes the spirals higher up, the greater is the energy expenditure, because the turns are larger than web part bellow the hub. According to this hypothesis, therefore, a larger increase from the hub to the edge in sticky spiral spacing and greater distances between spirals in the upper part compared to the lower part of the web is expected. Additionally, asymmetric webs with smaller upper parts are expected to be common, because building the upper part is energetically more expensive.

Functional hypotheses consider that edge-to-hub differences in sticky spiral spacing improve the foraging efficiency (Eberhard 2014). The first functional hypothesis, hereafter the "attack-time hypothesis," is based on the assumption that

spiders would invest more silk in web areas where they are more likely to capture prey successfully (Heiling & Herberstein 1998). Therefore, larger investment in sticky threads near the hub is expected because these locations can be reached faster by spiders. A higher investment in the lower part of the web is also expected because orb-weavers usually rest facing downward and run faster in this direction (Heiling & Herberstein 1998). This hypothesis is supported by the vertical asymmetry of several orb webs, which typically have a longer bottom part (below the hub) than top part (Heiling & Herberstein 1998; Herberstein & Heiling 1999; Zschokke 2002; Hesselberg 2010; Nakata 2012). The "radii-density hypothesis," on the other hand, suggests that spiders invest more silk in sticky spirals in segments with a greater capacity to stop prey. Therefore, the high investment in sticky spirals follows the density of radii (Zschokke 2002). Thus, a higher investment in sticky spirals near the hub and in the web part where radii are more parallel is expected. Finally, there are two additional hypotheses, the "sticky spiral entanglement" (Eberhard 2014) and the "prey tumbling" hypotheses (de Crespigny et al. 2001). The first is concerned with the idea that larger distances between sticky spirals near the edge may assure a lower chance of contact between adjacent loops because of the disturbance caused by the wind. The latter hypothesis states that smaller spaces between loops at the innermost area above the hub and outermost area below the hub of some spider species' webs could increase capture success when prey tend to escape by tumbling in vertical orb webs.

Tests of these hypotheses are restricted to a few common model species (Eberhard 2014). Therefore, the extension of evaluation to other orb web weavers must provide information on each hypothesis. In this study, we describe the orb web of *Wixia abdominalis* O. Pickard-Cambridge, 1882, and discuss the implications of web architecture in the scope of the functionality of its components. Stowe (1978) first described the unusual "asterisk" web of a *Wixia* species. According to him, the webs of *W. ectypa* (Walckenaer, 1841) presented a simple hub and typically only eight radii. Adhesive spirals were absent and spiders preyed exclusively on pedestrian arthropods. This adaptation to prey-specific capture is often mentioned in the literature on orb webs (Blackledge et al. 2011) and is attributed in general to *Wixia*. However, with the exception of *W. abdominalis*, all 49 species previously included in *Wixia* have been transferred to other genera, mostly to *Ocrepeira* Marx, 1883, and also to *Acacesia* Simon, 1895, *Alpaida* O. Pickard-Cambridge, 1889, *Wagneriana* F.O. Pickard-Cambridge, 1904, or considered as *nomen dubium* (*Wixia proxima* Mello-Leitão, 1940) (World Spider Catalog 2015). Data available in the literature on *W. abdominalis* are restricted to its morphology (Pickard-Cambridge 1882; Levi 1993) and to a few records of distribution in Guyana, Brazil (Pirenópolis, State of Goiás) and Bolivia (Levi 1993). Herein, we present the first record of the web pattern of the sole species remaining in the genus *Wixia*, showing that there is no impressive modification from the conventional orb web structure, such as those described in asterisk webs.

METHODS

Webs of *W. abdominalis* were located in a *Eucalyptus* plantation in Fazenda Nova Monte Carmelo (18°45′11″S,

47°51′28″W), Estrela do Sul, MG, Brazil. Areas covered by *Eucalyptus* were interspersed by fragments of Cerrado vegetation, but our surveys were restricted to regions within the monoculture. All webs were located at night during monthly expeditions and photographed after being lightly coated with cornstarch (see Eberhard 1976). Expeditions were conducted from August to December 2014 and surveys were restricted to the period from 19:00 to 0:00. All web measurements were performed from the photographs using the software ImageJ (National Institutes of Health, USA).

Web asymmetry (departure of shape of the web from a circle) and hub asymmetry (displacement of the hub from the web's geometric center) indices were evaluated according to Blackledge and Gillespie (2002). For the first index, positive values indicated vertically elongated webs. For the second index, positive values indicated upwardly eccentric webs. In both indices, values close to zero indicated symmetric webs.

Procedures to evaluate spacing of sticky spirals and radii were established following Eberhard (2014). We calculated the distance ratio between adjacent radii for each part of the webs, upper and lower. This ratio was obtained by dividing the distance between two adjacent radii at the outer edge by that of adjacent radii at the inner edge of the capture zone. We selected three pairs of adjacent radii for each part of the web (three pairs nearest to 0° and three pairs nearest to 180°), and used these to measure distance ratios. Then, we averaged the values for each part of the web. Ratios close to one indicate parallel radii. The distance ratios between adjacent radii in the upper and lower part of the webs were compared using a paired *t*-test.

We measured the distances between all the spiral loops on the most vertical radius for each part of the webs, upper and lower. These distances were standardized by dividing each measurement by the median value of distances on that radius. The standardized measure of distance between spiral loops was plotted against the relative distance to the hub, defined as the fraction of the number of spaces between loops attached to that radius. The value of one was closest to the hub. We conducted a linear regression analysis of standardized spaces between loops of sticky spirals and the relative distance from edge-to-hub. In this regression, we excluded 20% of these standardized spaces in the inner and outer portions of the capture zone and used the 60% in the middle portions. We used this approach, because these innermost and outermost portions have spaces between spiral loops especially smaller or greater in many species, generating non-linear data (Eberhard 2014). We compared the spaces of these portions with the middle portion, using Friedman paired tests and Nemenyi *post-hoc* tests (see Pereira et al. 2015) by means of the 'PMCMR' R package (Pohlert 2014). We performed data analysis in R software, version 3.3.2 (R Development Core Team 2016).

Previously, we observed that some individuals constructed webs with a free-sector. These spiders remain out of the hub, holding a signal line connected to the hub from a position on a branch of *Eucalyptus* (Fig. 1D). The presence of free-sectors may imply behavioral changes during prey capture and may impose constraints on other web components. Hence, we evaluated webs with and without free-sectors separately. We performed analyses of covariance (ANCOVA) in order to
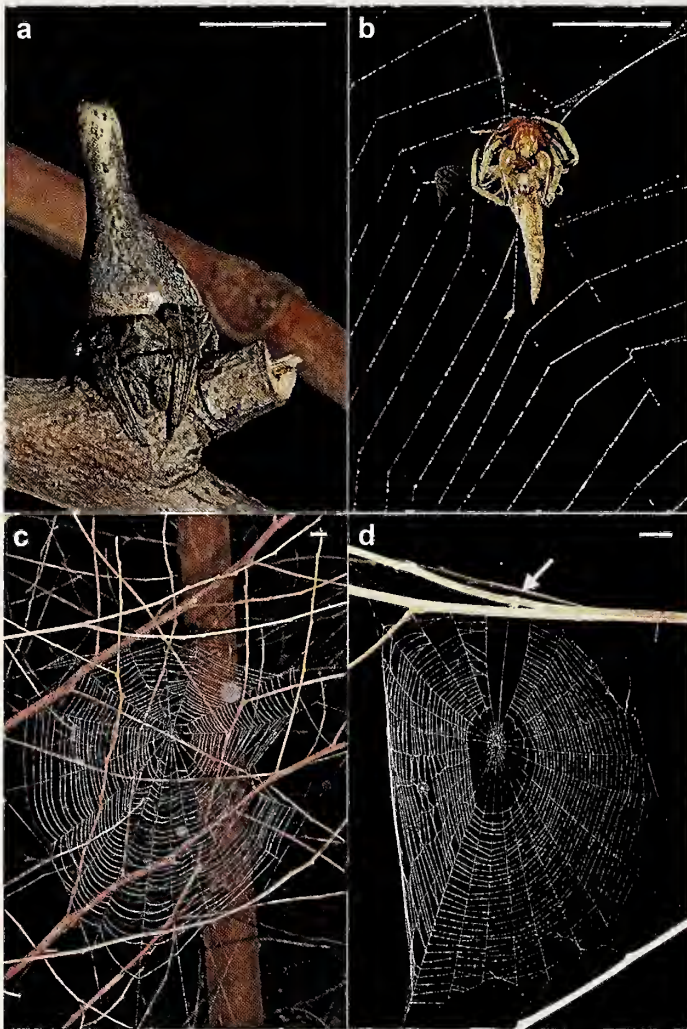
Figure 1.—*Wixia abdominalis*. (A) Resting position on an *Eucalyptus* branch. (B) Subadult male capturing a termite. (C) Web without free sector. (D) Web with a free-sector. The arrow indicates the spider position. Scales: A, B: 5mm; C, D: 1cm.

assess if: (1) the linear regressions differ between webs with and without free-sector, (2) the linear regressions differ between upper and lower part of all webs. To use parametric tests, we assessed the assumptions of normality and homoscedasticity by means of graphical analysis of residual distribution.

RESULTS

Individuals of *W. abdominalis* are active nocturnally, ingesting their webs at dawn. During the day, the spiders remained motionless in a cryptic position on the branches of *Eucalyptus* trees (Fig. 1A). In this position, they resembled a piece of broken wood.

We recorded webs of 28 juvenile individuals (all at antepenultimate or penultimate instars, weighing 0.024 ± 0.007g, mean ± se, min. 0.0059g and max. 0.1819g) and two adult females (weighing 0.047 and 0.074g). All webs appear to be more or less vertically oriented (Fig. 1B–D). Some webs spun by juveniles included a free-sector ($n = 16$). The angle of

signal lines in the free sectors deviated slightly from vertical orientation (16.69° ± 4.02, mean ± se) . In these webs, spiders remained on a branch located immediately above the orb at night, holding a thread connected to the web hub. In webs without a free-sector ($n = 14$), spiders waited for prey interception at the hub, facing downward. However, the frequency of these behaviors was not quantified herein. Webs with and without free-sectors were analyzed separately, because there was no information on how or whether this variation in orb design would influence other web parameters.

Juvenile individuals built slightly vertically asymmetric webs (web asymmetry index = 0.119 ± 0.03) and webs with hubs were displaced upward (hub asymmetry index = 0.325 ± 0.03). The two webs built by adults were more vertically asymmetric (web asymmetry index = 0.328 and 0.273), and their hubs were displaced upward (hub asymmetry index = 0.494 and 0.129).

From the analysis of the webs of immature and adults pooled, the average distance ratio between adjacent radii was 7.15 ± 0.404 in the upper part and 5.96 ± 0.45 in the lower part of the webs ($n = 29$). The upper part had greater ratios than the lower part of the webs (paired t-test: $t = 2.818$, $P = 0.009$). Therefore, in the upper part, the radii were less parallel and the difference in radii density between inner and outer portions was more pronounced than in the lower part of the webs. Webs with and without free-sectors were not different in distance ratios between adjacent radii in the upper (t-test: $t = -1.569$, $P = 0.128$) or lower part of the webs (t-test: $t = -1.753$, $P = 0.091$).

The tendency of increasing sticky spiral spacing from the hub to the edge of the capture zone was confirmed (Tables 1, 2, Fig. 2). However, when only webs with free-sectors were analyzed, a uniform pattern of sticky spiral spacing in the lower part was detected (Table 2, Fig. 2). In the upper part of the webs, the increase of sticky spiral from the hub to the edge was more accentuated in webs with free-sectors than webs without free-sectors (ANCOVA: $F_{1,835} = 6.882$, $P < 0.01$). In the lower part of webs, the increase of sticky spiral spacing from the hub to the edge was more accentuated in webs without than webs with free-sectors (ANCOVA: $F_{1,360} = 7.108$, $P < 0.01$). In general, the increase in sticky spiral spacing from the hub to the edge was more accentuated in the upper part than in lower part of the webs (ANCOVA: $F_{1,119} = 30.601$, $P < 0.001$), but the 20% outermost distances between sticky spirals in the upper and lower web parts were similar ($t = 0.410$, df = 26, $P = 0.685$).

DISCUSSION

Wixia abdominalis webs were vertically elongated with upwardly displaced hubs. The radii were always less parallel in the upper part and there was increasing sticky spiral spacing from the hub to the edge of the capture zone. The increase was larger in the upper part. However, webs presenting a free-sector exhibited uniform spiral spacing. Despite that, we observed a similar enlargement in the 20% outermost spacing in both parts of the webs. These results did not fit well in any single hypothesis proposed to explain differential investment in distinct web segments. Based on our results, more than one hypothesis could be used to explain web architecture of *W. abdominalis*.

Table 1.—Analyses of differences in the sticky spiral standardized spaces between the portions of orbs: the outer (0–20% of spirals from the edge to the hub), the middle (20–80%) and the inner (80–100%). We assessed the difference between the means of standardized spaces of each web portion using Friedman paired tests. The letters indicate differences between orb portions in the Nemenyi *post-hoc* tests with $P < 0.05$, and n corresponds to the number of webs.

Web	n	Means ± SD			Friedman test	
		Outer	Middle	Inner	χ^2	P
Upper						
With free-sector	16	1.55 ± 0.70 a	1.05 ± 0.45 b	0.85 ± 0.37 b	22.87	< 0.05
Without free-sector	11	1.30 ± 0.66	1.03 ± 0.32	1.02 ± 0.55	3.81	0.14
All	27	1.45 ± 0.70 a	1.04 ± 0.40 b	0.92 ± 0.46 b	24.22	< 0.01
Lower						
With free-sector	16	1.28 ± 0.70 a	1.01 ± 0.38 b	1.15 ± 0.49 ab	7.87	< 0.05
Without free-sector	11	1.43 ± 0.71 a	1.03 ± 0.35 b	1.03 ± 0.45 b	13.16	< 0.01
All	27	1.34 ± 0.71 a	1.01 ± 0.37 b	1.11 ± 0.47 ab	18.28	< 0.01

According to Peters' segment rule, we would expect sticky spiral spacing to increase from the hub to the edge. In addition, this increase would be greater for segments adjacent to less parallel radii (at the upper part). However, we observed that the 20% innermost spirals of webs were not less spaced than the 60% spirals of the middle part. Another pattern that contradicts Peters' segment rule is the uniform pattern of sticky spiral spacing in the lower part of the web of *W. abdominalis*. Similarly, spiral spacing of other species, including *Nephila clavipes* (Linnaeus, 1767), *Metepeira* sp. F.O. Pickard-Cambridge, 1903, (Eberhard 2014), *Argiope keyserlingi* Karsch, 1878, (de Crespigny et al. 2001), and some *Cyclosa* species Menge, 1866, (Zschokke & Nakata 2015), cannot be explained by this incidental hypothesis.

The "energetic constraint" incidental hypothesis, on the other hand, was partially corroborated by the greater increase in sticky spiral spacing from the hub to the edge in the upper part. However, at the higher distances at the outer segment of the upper part this would be expected. Instead, the webs of *W. abdominalis* showed similar distances between sticky spirals at outer edges of upper and lower web parts. Despite this contradiction, the asymmetric webs observed in *W. abdominalis* and in other species (Herberstein & Heiling 1999) suggest some degree of energetic constraint during web building. Indeed, it was found that spiders spent more energy building the upper part of vertical orb webs than the lower part (Coslovsky & Zschokke 2009). However, it is difficult to accept that variation in sticky spirals could be determined solely by energetic constraints during web building, especially

when accounting for the functional value of the uneven distribution of silk (Masters & Moffat 1983).

The greater investment in sticky spirals near the hub and in the lower part of the web is in accordance with the "attack-time hypothesis" (Heiling & Herberstein 1998). *Araneus diadematus* Clerck, 1757, for example, presents a similar web pattern, with spirals evenly spaced below the hub and it can indeed run faster downward (ap Rhisiart & Vollrath 1994). However, *W. abdominalis* has a special condition regarding spider movements during prey capture events. Individuals with a free-sector in their webs remain on the branch located above the orb, holding a signal line, whereas individuals with complete orbs wait at the hub. In the first case, after the detection of movements of an entangled prey, the spider moves first from the resting position at the twig to the hub and then goes to the location of the prey on the web. Therefore, the spider moves for a longer distance to reach the prey. We observed that webs with free-sectors presented a pattern of spacing in the lower part that was more homogeneous than the pattern of the webs without free-sectors. This architecture is not congruous with the scope of "attack-time hypothesis", because spiders are investing in retention further away from themselves. It may be a compensatory mechanism for spiders that rest out of web. Thus, it occurs just in lower part because it is the web part where spiders reach faster. This "compensation hypothesis" provides a theoretic understanding of the function of the uniform distribution of spirals at the lower part of webs with free-sectors; however, behavioral observations during prey capture events are still needed.

Table 2.—Linear regression analyses of standardized sticky spiral spacing and the relative distance from the edge to the hub in the middle portion of the capture zone (between 0.2 and 0.8).

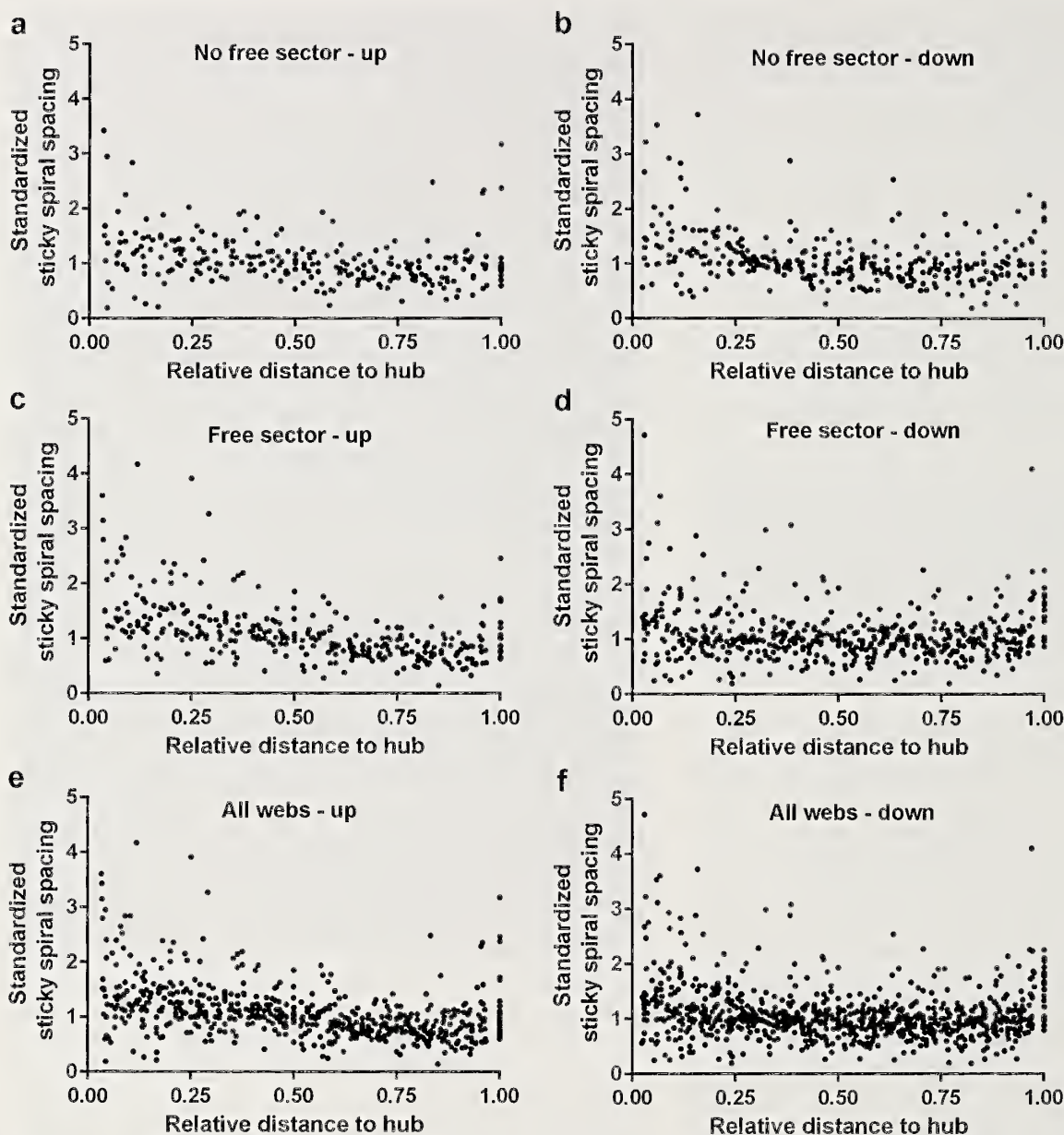| Web part	R^2	F	DF	Equation	P
Upper					
With free sector	0.216	54.921	1, 199	$y = -1.223x + 1.668$	<0.001
Without free sector	0.167	29.383	1, 147	$y = -0.755x + 1.406$	<0.001
All	0.192	82.915	1, 348	$y = -0.02x + 1.554$	<0.001
Lower					
With free sector	0.010	2.864	1, 298	$y = -0.217x + 1.114$	0.092
Without free sector	0.065	14.554	1, 211	$y = -0.478x + 1.25$	<0.001
All	0.025	13.149	1, 511	$y = -0.33x + 1.174$	<0.001

Figure 2.—Edge-to-hub patterns of relative standardized sticky spiral spacing in orb webs of *Wixia abdominalis*. 'Relative distance to the hub' was calculated following Eberhard (2014) and is defined as the ratio between the 'number of sticky loops between the site in the orb and the outer edge of the capture zone' and 'total number of sticky spiral loops from the outer edge to the hub'. The value 1 is the closest to the hub.

The "radii-density hypothesis" was also corroborated by our data, because there was a higher increase in sticky spiral spacing from the hub to the edge in the upper part of the webs, where radii were less parallel. However, according to this hypothesis, we would expect heterogeneity in the pattern of spiral distribution in the lower part of the webs with free-sectors. Similarly, the spiral spacing of other species is also not in complete accordance with this hypothesis, for example, *Nephila clavipes* and *Metepeira* sp., have a higher density of spirals at the edge of their orbs and adopt other strategies to increase the absorption of their prey's kinetic energy, such as the maintenance of auxiliary spirals and division of radii, increasing radii density toward the middle and outer edges of the web (Eberhard 2014). However, these auxiliary structures to absorb kinetic energy are absent in webs of *W. abdominalis*.

Therefore, the uniform pattern of spiral distribution in the lower part of the webs with a free-sector may be a result of an interaction between absorbing kinetic energy (Zschokke 2002) and compensating for the longer distance to the interception site (Heiling & Herberstein 1998).

The "sticky spiral entanglement" hypothesis is contradicted by the observation that spiral spacing was not significantly greater at the edge above the hub than at the edge below the hub. Some spiders may use alternative strategies to reduce web damage from the wind, softening the constraints imposed on its web architecture, such as the reduction of sagging of threads making them stiffer under windy conditions (Vollrath et al. 1997) and changing orb web orientation according to wind direction (Hieber 1984). The "prey tumbling" hypothesis also was not supported by our data, because there was an

enlargement in spiral spacing on the outer edge of the lower part of the webs. However, prey tumbling is highly variable depending on web adhesiveness and prey identity (Zschokke et al. 2006).

In the present study, we recorded the web structure of *W. abdominalis* and observed that it differed dramatically from the patterns previously attributed to the genus *Wixia*. The architecture of the *W. abdominalis* web allowed us to discuss several hypotheses based on differential investment in distinct parts of the web as it applied to the observed characteristics. The web architecture of *Wixia abdominalis* was better explained by the "energetic constraint" incidental hypothesis (Herberstein & Heiling 1999), and "attack time" and "radii density" functional hypotheses (Heiling & Herberstein 1998; Zschokke 2002). Our results are in accordance with the findings of Eberhard (2014) and Zschokke and Nakata (2015) in that different parts of the orb web present different properties and functions. The analysis of interspecific variation in orb web designs and the association of these designs with aspects of the natural history of each species are important to understand the conditions determining each pattern. In addition, our study showed there was relevant intraspecific variation in certain web traits (such as inclusion of a free-sector) that might also influence web design.

ACKNOWLEDGMENTS

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Natural history and courtship behavior in *Tengella perfuga* Dahl, 1901 (Araneae: Zoropsidae)

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Abstract.   *Tengella perfuga* Dahl, 1901 is a Nicaraguan cribellate zoropsid spider found in high altitude remnant cloud forest habitats bordering coffee plantations. Since its description in 1901, and its rediscovery in 2012, almost nothing is known of its natural history, life history, courtship or web spinning behavior. Observations were made in the field, as well as in the lab. Mature female *T. perfuga* occurred in funnel webs with several knockdown lines comprised of cribellate silk, and that were typically placed between buttress roots of strangler figs or other outcropping structures, while males abandoned their webs upon adulthood to search for females. Here, we describe the life history, growth, web ontogeny, courtship and reproductive behaviors, as well as silk use of this spider for the first time. There are 11–12 instars to reach adulthood and cribellate silk did not appear in juvenile webs until the eighth instar. Interestingly, orbicularian-like behaviors were observed in the initial appearance of cribellate silk lines in the juvenile web in a spiral-like pattern. Males exhibited positive allometric growth in Leg 1 from penultimate to adult instars, which likely plays an important role in courtship; this included strumming the sheet, stroking the female and depositing a thin 'bridal veil' of silk on the female. Virgin females had 'mating plugs' prior to exposure to males. This suggests that *T. perfuga* may be an interesting species with which to further examine sexual evolution and female choice.

Keywords: Web ontogeny, growth, cribellate silk, reproduction

Tengella perfuga Dahl, 1901 is the type species for the genus *Tengella* Dahl, 1901, which is the type genus for the recently reassigned family Tengellidae Dahl, 1908 (= Zoropsidae Bertkau, 1882 (Polotow et al. 2015)). They are medium to large cribellate spiders and little is known about their natural history, courtship behavior, feeding behavior and web ontogeny (Fig. 1, Platnick 2009; Leister et al. 2013). While spiders of the genus *Tengella* are distributed across various habitats in Central America, from caves in Mexico to tropical lowland forests in Panama, *T. perfuga* is limited to high altitude remnant cloud forest habitats bordering coffee plantations in northwestern Nicaragua and Honduras (Leister et al. 2013, S. Longhorn, pers. comm.). However, the potential sister species, *Tengella radiata* (Kulczyński, 1909), is widespread and found in various high and low elevation tropical forests, coffee plantations and developed sites from Honduras south to Panama (Leister et al. 2013). The phylogenetic placement of *T. perfuga* has long been uncertain in large part due to its use of cribellate silk (Fig. 1). Cribellate silk is produced from glands that open on a plate-like structure, the cribellum, derived from the ancestrally lost anterior median spinnerets (Pechmann et al. 2010). Cribellate silk production also requires a comb (calamistrum) on the fourth leg which primes the silk glands and combs out the loops of fibrils (Hawthorn & Opell 2002). Use of this type of silk in *Tengella* is surprising, given the developmental investment and metabolic costs to maintaining the cribellum and calamistrum (Blackledge et al. 2009a), and that this trait is typically found in older spider lineages, whereas *Tengella* is part of a more recently evolved clade, the majority of whose members have evolved the loss of silk use as a foraging tool (Raven & Stumkat 2003, 2005; Griswold et al. 2005; Spagna & Gillespie 2008; Blackledge et al. 2009a, b; Agnarsson et al. 2013; Polotow et al. 2015). As such, *T. perfuga* is at the crux of important phylogenetic questions about the evolution of silk use in spiders, particularly within the RTA clade to which both *Tengella* (zoropsids) and their sister group Lycosoidea belong (Griswold et al. 2005; Polotow et al. 2015).

One approach to understanding the evolutionary history of silk use is to observe web ontogeny. Early instar webs may resemble the ancestral web design or plesiomorphic traits in silk use, so understanding web ontogeny can help reconstruct evolutionary history (Eberhard 1985, 1986; Barrantes & Madrigal-Brenes 2008; Barrantes & Eberhard 2010). Given the recent genomic evidence of paraphyly in the Orbiculariae and the placement of *T. perfuga* within the RTA clade, a web ontogeny study becomes important as a way to behaviorally examine the evolution of silk use and orb web ancestry in this clade (Agnarsson et al. 2013; Bond et al. 2014; Fernandez et al. 2014). Orbicularian traits of silk use within the RTA clade have only recently been examined once, where the phylogenetic placement of the Psechridae, a family of cribellate pseudo-orb weavers, was moved from the Orbiculariae to the RTA clade (Agnarsson et al. 2013). This study of *T. perfuga* is the first study to specifically look for evidence of orbicularian behavioral traits in a member of the RTA clade.

Two previous studies were conducted on *T. radiata* and these served as a basis for the studies reported here. *Tengella radiata* develop to adulthood in 9–10 molts, with males having longer forelegs than females (Barrantes & Madrigal-Brenes 2008). Webs begin as basic sheets and more complex elements are added with each instar; notably, cribellate silk does not appear until the seventh instar (Barrantes & Madrigal-Brenes 2008). Males court females with a series of plucking the web, rocking motions and tapping to induce the female to copulate; females show some aggressive reactions to potentially unsuitable partners (Barrantes 2008). Males also exhibited a 'flub' behavior while attempting insertions, with repeated scraping motions, but successful insertion and single expansion of the hematodocha was extremely rapid when it occurred (Barrantes 2008).

The objective of this study was to learn more about these enigmatic spiders, particularly their use of cribellate silk, life history, behavior, and ontogenetic changes in web size and structure.

METHODS

Field collection and specimen sources.—Live *T. perfuga* adult females were collected in Nicaragua (Selva Negra, 12.9984°N,
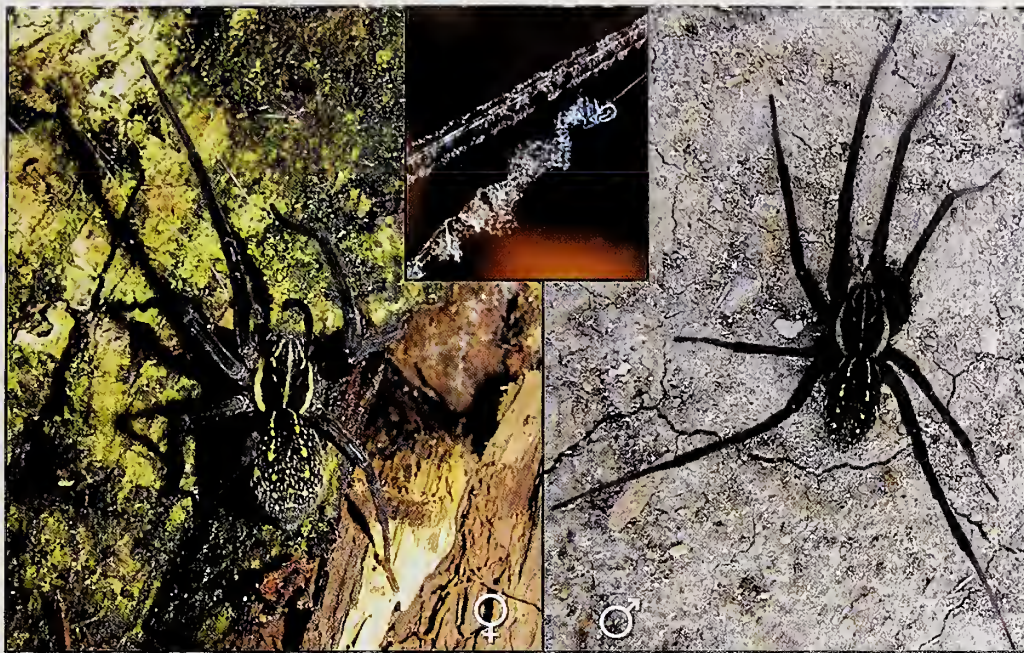
Figure 1.—Adult male (right) and female (left) *Tengella perfuga*, with cribellate silk from a female web (inset) (Spider whole body photos: M. Leister, with R. Mallis 2012).

85.9105°W) in May 2012 (permit: DGPN / DB – 09 – 2012), and subsequently in May 2014 (permit: DGPN / DB – 006 – 2014) and allowed to mate and/or lay egg sacs, giving rise to a lab-reared spider colony which at one time numbered well over 500 individuals. Many of these reached adulthood and reproduced, allowing for observations of courtship behavior, growth and web ontogeny. Observations of web structure and feeding behavior were made in the field ($n \gg 100$). Most field encountered males were collected in female webs (in 2014, $n = 7$; in 2012, $n > 15$) or as penultimate males in their own webs. All field-caught spiders and reared individuals are deposited in the Museum of Southwestern Biology, Division of Arthropods collection or teaching collection (MSBA 24980 — 24982, 24985, 24986, 29081, 29082, 30589, 30591, 30592, 30596 — 30599, 30619, 30621, 30622, 30635, 30640, 30642, 30643). A complete instar series, as well as SEM specimens, from second instar to adult male and female *T. perfuga* is also deposited at the California Academy of Sciences, Department of Entomology alcohol collection. These include a few of the specimens used in the growth and web ontogeny studies.

Web ontogeny and life history.—A sub-group of approximately 50 second-instar individuals, which had recently emerged from two different egg sacs, were removed from the maternal webs and housed individually. Web ontogeny and spider growth at each instar stage were measured. Each stage is referred to by its sequential number; for example, the second instar is called 'instar 2' and so forth. We measured the width at widest points and the lengths of the cephalothorax, femur I, tibia I and the overall body length of recently molted individuals for each instar. Measurements were made using an Olympus SZ60 binocular dissecting scope equipped with a calibrated 10× micrometer. Images of instars and silk were taken using a Visionary Digital System (online at http://www.visionarydigital.com). We noted web dimensions and characteristics such as the appearance of cribellate silk, but the webs of older instars filled

the containers in which they were housed (Gladware® storage containers, 15.5×15.5 cm and approximately 3.5 cm high) and so web size was artificially limited. Webs for each instar were imaged using a Nikon Coolpix L110 camera.

Colony Maintenance: Spider habitats were Gladware® square plastic storage containers filled with a layer of EcoEarth®. Similar to a study of the closely related *T. radiata*, we provided 2–4 pieces of corkwood for web attachment, rather than rocks, and a retreat option of a 2mL vial, rather than a rolled-up leaf as in a previous study (Barrantes & Madrigal-Brenes 2008). In 2012, we collected two gravid females in the field, and used 25 spiderlings from one female and 27 from the other in our study. Three groups of six additional spiderlings each were set up for observation as well to better understand conspecific tolerance, as *T. perfuga* was sometimes found at higher densities in a single site in the field. Containers were spritzed with distilled water weekly to provide moisture, and spiders were fed a steady diet of crickets ranging in size from pinheads to medium-large and fruit flies. Natural history traits such as feeding behavior and timing of molts were recorded. Containers were monitored daily for spider status and spiderlings fed twice weekly, but as they aged, feedings became once weekly to biweekly. Spiders were maintained at ambient room temperatures in the laboratory and as close to a 10:14 light:dark ratio as possible. Individuals that died of natural causes were also preserved in 70% EtOH with a leg placed in 95% EtOH for potential future molecular sequencing work.

Measurements: After all spiders in the colonies had completed an instar and molted, three from a pool of the two mothers' offspring were randomly selected and their containers placed in a -20°C freezer to preserve the web for imaging. Culled spiders were immediately removed after webs were imaged. One spider was stored in 95% EtOH at -80°C to preserve genetic data, and the remaining two were placed in 70% EtOH and used for morphological measurements. Webs were photographed dry

Figure 2A–C.—Webs in the field in Nicaragua, showing variation in structure. A. Preferred habitat of strangler fig buttress roots. B. stacked webs of juveniles. C. adult female web.

and subsequently wet after being lightly spritzed with water to increase their visibility, and the presence or absence of cribellate silk and length, width and height (if applicable) measurements were taken. Webs from eighth-instar spiders were inadvertently damaged prior to measurements, but presence or absence of cribellate silk observations were still recorded.

Averages and standard deviations of morphological measurements for each instar were calculated from the pooled data per instar. To calculate the relative percent growth from instar to instar, we used the equation as in Barrantes & Madrigal-Brenes (2008): [(Tibia I InstarN – Tibia I InstarN-1)/Tibia I InstarN-1] * 100. While much of our approach for the natural history study was inspired by Barrantes & Madrigal-Brenes (2008), we had some differences, such as spritzing webs with water rather than corn starch and more importantly, using

culled spiders for measurements, rather than rehydrating shed molts from the same individual spiders as they grew.

Courtship observations.—Using adult spiders from later generations and other field collections, as well as the remaining adults from the web ontogeny study, non-related pairs were randomly assigned for mating ($n = 35$ documented observations; 24 initial pairings, and 11 subsequent interactions). All females were virgin and well fed prior to introduction of the males. Males were placed onto female webs and courtship encounters were video recorded and behaviors noted. Males were removed after copulating once or after rejection by the female in order to reduce the chances of mortality ($n = 24$). They were secondarily introduced to the same or a different non-related female to propagate the spider colony, but allowed us to observe differences in courtship behavioral patterns and acceptance ($n = 11$). Females were later allowed to lay egg sacs following successful copulations.

RESULTS

Webs in the field.—*T. perfuga* sheet webs typically had a funnel retreat either at a corner or the middle of the sheet that receded into the substrate ($n \gg 100$) (Fig. 2). Sheets were typically surrounded by a scaffolding of tangle or knockdown lines above and anchor lines below (Fig. 2; Leister et al. 2013). The sheets were lined with cribellate silk and the majority of knockdown lines had cribellate silk laid over them. Webs typically occurred along stream embankments, tree trunks (especially strangler figs) and between stones or wood beams on structures at high elevation cloud forest sites associated with shade coffee plantations in northern Nicaragua. Spiders were observed in the retreat or just at the retreat opening; they ran out onto the sheet to capture prey and drag it back to the retreat, as we also recorded in Leister et al. (2013).

A variety of web locations were noted in the field, with some adult female webs appearing in unexpected places, like a hole in a trail sign on a tree or across the span of an empty bell tower of a stone chapel. Some webs had egg sacs or second or third instar spiderlings in the retreat (Fig. 3A, B). The egg sacs were covered with pieces of the surrounding substrate, from bark to soil to leaves (Fig. 3B). Egg sac production was not observed in the field. At two less disturbed sites, webs were
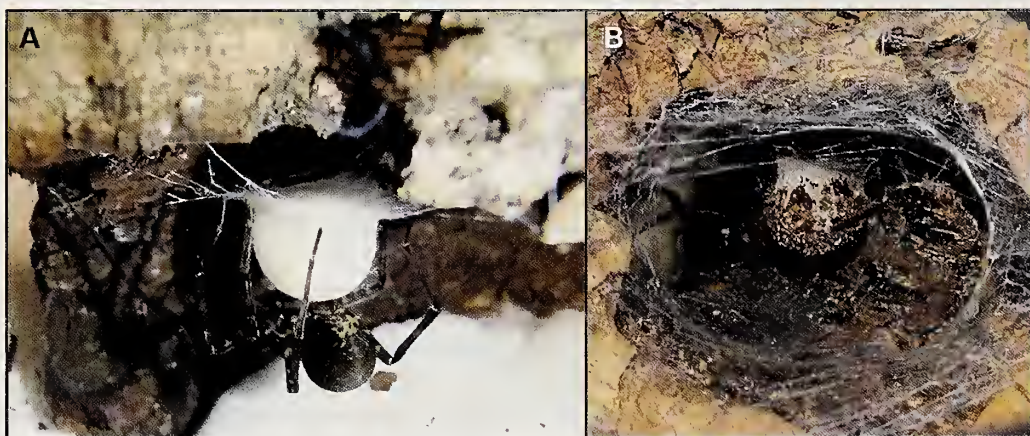

Figure 3A–B.—Egg sac construction and camouflage. A. Female silking over egg sac after depositing the eggs. B. Female guarding a camouflaged egg sac.
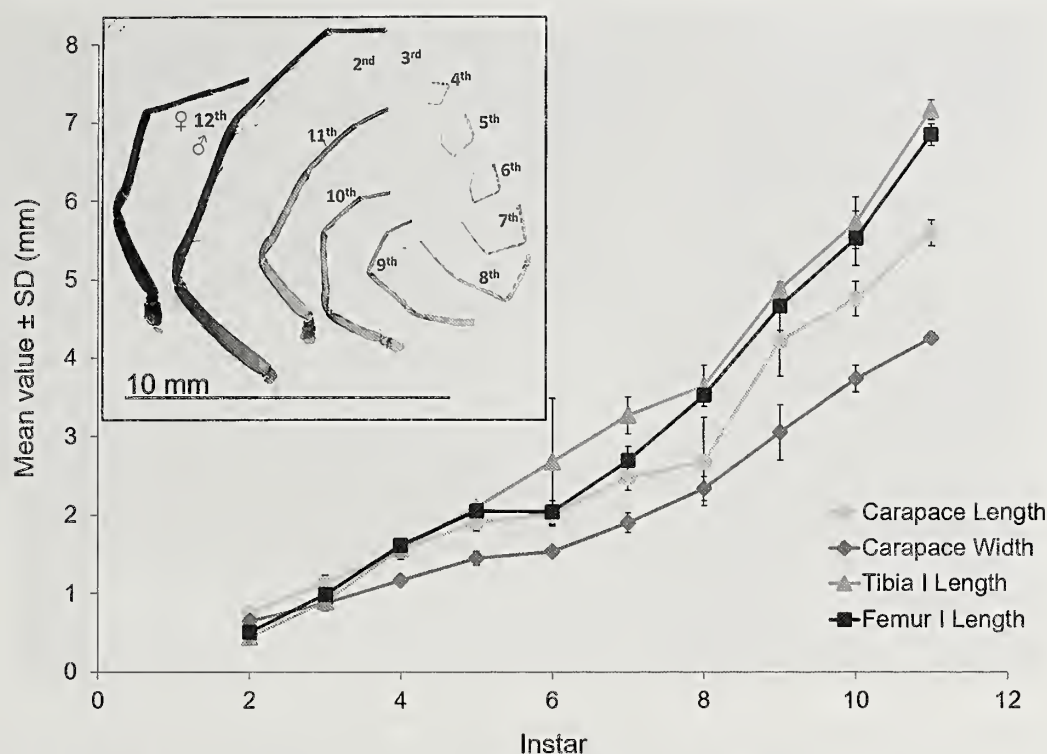
Figure 4.—Growth from instar to instar of cephalothorax length and width, femur I and tibia I lengths. Comparison of the amount of growth in Leg I that occurs from second to twelfth instar (adulthood), as well as the allometric growth of male during the final molt (inset).

observed with commensal bugs and kleptoparasitie spiders, similar to reports for the closely related species, *T. radiata* (Eberhard et al. 1993). These web symbionts have not yet been identified.

Observations in the laboratory.—*Tengella perfuga* reached adulthood between 205 – 226 days and 11–12 molts after emerging from the egg sac as a second instar nymph. Females reached adulthood typically in 12, sometimes 11 instars, whereas most males reached adulthood in 10–11 instars. For the morphological measurements and web ontogeny observations, we used males who reached adulthood in 12 instars. Female *T. perfuga* tended to be more robust than males, however the color patterning is similar (Fig. 1). Males had longer legs than females, particularly leg I (Fig. 4 inset; Leister et al. 2013). The calamistrum did not appear to be a full oval until instar 5, and the cribellum did not appear to be functional until instars 7 and 8 (see Fig. 5). Cribellate silk did not appear in the webs until the eighth instar (Figs. 6 & 7). After hatching from the egg sac, spiderlings remained with the mother in her retreat on a collectively spun 'molting web.' Once molted to the third instar, spiderlings began to disperse.

Most of the early instar webs exhibited features such as a simple sheet and clear retreat tunnel similar to those of adult webs, but were smaller and lacked cribellate silk (Fig. 6A, B). First, a tiny retreat was formed with some lines extending to form the scaffolding for the subsequent sheet (instar 3), then a sheet was filled in (instar 4). Prey capture was still sueeessful, despite a small capture surface, and these instars grabbed prey through the retreat or sheet or ran on top of the structure to bite the prey. Knockdown lines were not observed until instars 5 and 6 (Fig. 6A, B). When cribellate silk first appeared in the webs, it was in an orb-like spiral laid out on the sheet (Fig. 7).

Ultimately, adult webs were comprised of a deep retreat into the substrate, surrounded by a broad sheet with several knockdown lines or 'scaffolding' above the sheet and retreat entrance. The majority ($n \sim 25$ adult webs observed) of the structures were lined with eribellate silk (Figs. 2 & 6D).

Life history: Eggs hatched in approximately 54 days ($n = 2$ egg sacs). It took five days for all spiderlings to leave the egg sac. In the groups of six spiders, each spider in the group had its own retreat and shared use of the sheet. Minimal cannibalism was observed and there appeared to be tolerance for con-specifics. Development time varied. Small males were observed after 9 – 10 molts. Some females developed with less time between each of the 12 molts. Other members ($n = 4$) of the groups appeared to have arrested development in earlier instars and never reached adulthood during the nearly year-long observational period.

Growth: Overall, from second to eleventh instar, *T. perfuga* grew by 612% relative to the body length of a second instar (Table 1). Other body parts, such as tibia I and femur I, also grew by over 1400% and 1200%, respectively (Table 1; Fig. 4). There appeared to be less growth between the fifth and sixth instars and seventh and eighth instars (Table 1, Fig. 4). Because the sample size for each instar ($n = 2$–3) was small, there was no power for statistical analyses. Second instar spiders did not eat, as they still had yolk fat, and although they were able to readily walk on the mother's web, the legs appeared short for the body size (approximately 4:1 ratio of body length to tibia I length, vs. approximately 2:1 ratio of body length to tibia I length in penultimates). Spiders began foraging on their webs in the third instar, and this was when their overall appearance mirrored adult gestalt and pattern. Males had a greater increase in leg I length than females from
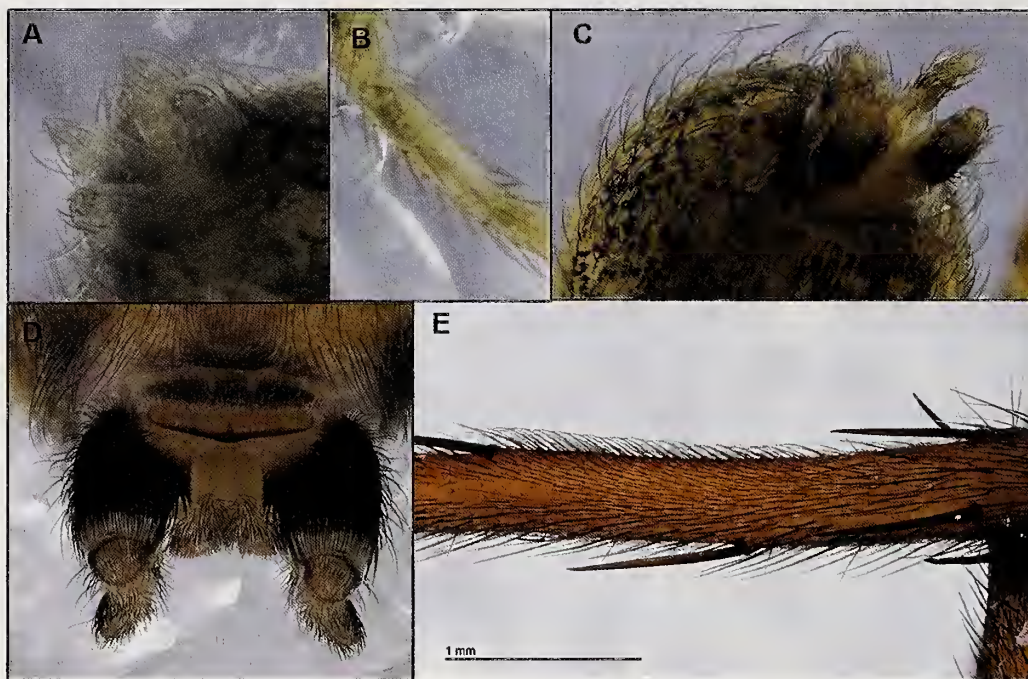
Figure 5A–E.—Cribellum and calamistrum images from selected instars demonstrating increasing size and complexity of structures, as well as potential functionality. A. Instar 2, note the lack of a cribellar plate. B. Instar 2, leg IV, note the lack of the calamistrum (however, see next image). C. Instar 3, cribellum present. D. Instar 11, cribellum (penultimate male). and E. Instar 11, oval shaped calamistrum (penultimate male).

Figure 6A–D.—Web images from selected instars in the lab, demonstrating web growth and increasing complexity of structure and the presence of cribellate silk. A. Web of third instar spiderling. B. Web of fourth instar spiderling. C. Web of ninth instar spiderling. D. Web of adult female with male present (Instar 12).

were observed in the web, with some seemingly thinner and other 'combed out' areas filling in the webs (Fig. 6C, D). The cribellum appeared functional (fully developed) in instar 7. The calamistrum appeared functional between instars 5 and 6, during which little body growth occurred (Table 1, Fig. 4).

Second instar spiderlings did not possess a cribellar plate-like structure or any precursors to functional spigots, nor did they have a calamistrum or any type of modified setae on metatarsus IV (Mallis-Alfaro, Miller & Griswold, unpublished data). Third instar spiders possessed a single row of stout curved setae as a calamistrum and a small cribellum. Despite cribellar spigots present from third instar onward to adult (instars 11/12), and active expansion of the spigot field on the cribellum from instar to instar (Mallis-Alfaro, Miller & Griswold, unpublished data), functionality did not seem to occur until the appearance of cribellate silk in the eighth instar. Similarly, the calamistrum expanded from a single row of setae to an oval shaped patch in the sixth instar that appeared as in the adult. Under the dissecting microscope, the cribellum appeared as a pseudobipartite plate from the third instar onward. There was a single cribellar plate, but two separate spinning fields of cribellar spigots in third and subsequent instars (Mallis-Alfaro, Miller & Griswold, unpublished data).

To comb the cribellate silk out, spiders crossed both the 'combing leg' (leg IV) and the 'supporting leg' (other leg IV) and moved them synchronously as a single unit, using swift and sharp anterior to posterior movements. The tarsus of the combing leg IV rested on the lower half of the metatarsus on the supporting fourth leg. The same combing legs were used to complete each cribellate silk segment being laid down. Spiders tended to switch combing legs between cribellate lines.

Ontogenetic changes in web structure: Second instar spiderlings emerging from the egg sac did not construct individual webs. Instead they remained in the maternal retreat on a molting web spun collectively by all spiderlings. The molting web was comprised of thin drag lines with no adult silk contributions; however, movement onto and throughout the mother's web was possible. Third instars began to disperse from the maternal web to form individual webs. In three cases, two in the field, one in the lab, some formed a second collective web separated from the mother's retreat in the tangle scaffolding. In the webs of third instar spiderlings, the

Figure 7.—The orb-like pattern, indicated by the arrows, of cribellate silk in the sheet of a juvenile, eighth instar *T. perfuga*. In this image, focus was sharpened and contrast was enhanced in order to aid in observing the spiraling cribellate lines.

the penultimate to adult molt, going from an average of tibia I length of 7.17 mm to 11.19 mm in the male versus 7.17 mm to 7.63 mm in the female, suggesting allometric growth occurred (see Fig. 4 inset, adult length values previously published in Leister et al. 2013).

Ontogeny of the cribellum, calamistrum and cribellate silk use: In mature individuals of both sexes, the cribellum was a pseudobipartite plate (Fig. 5D). There were two patches of spigots in the female, a row of setae at the anterior margin and a line of sclerotization at the posterior margin. The calamistrum was an oval patch that extended one third the length of metatarsus IV on the proximal half dorsoretrolaterally (Fig. 5E). The male cribellum was a featureless plate; however, he retained a calamistrum. Cribellate silk appeared in the webs between instars 7 and 8, first lining the retreats or incorporated in the tangle above the sheet, and ultimately in the sheet itself in an orb-like spiral radiating out from the retreat, replacing main support lines, then filling in the sheet in subsequent instars (Figs. 6 & 7). Mostly thick cribellate lines

Table 1.—Instar growth data, using the averages of 2–3 spiderlings and the standard measures of carapace length and width, tibia I length and width, femur I length and width and body length (all in mm). In order to calculate the relative percent growth from instar to instar the same equation as Barrantes & Madrigal-Brenes (2008) was used to calculate relative percent growth from instar to instar: [(Tibia I InstarN – Tibia I InstarN-1)/Tibia I InstarN-1]* 100 (Standard deviations not listed here).

| Instar	Percentage Change Between Instars						
	Carapace Length	Carapace Width	Tibia I Length	Tibia I Width	Femur I Length	Femur I Width	Body Length
2 to 3	48.68	34.62	102.25	41.18	94.12	71.43	35.73
3 to 4	37.17	32.57	74.44	33.33	63.64	8.33	39.88
4 to 5	23.55	25.00	33.44	34.38	26.54	48.72	24.44
5 to 6	3.92	5.52	27.92	16.28	-0.49	0.00	12.87
6 to 7	24.37	24.18	21.83	10.00	32.11	18.97	25.10
7 to 8	8.28	22.89	11.64	9.09	30.80	42.03	19.90
8 to 9	57.46	30.62	33.88	65.00	32.20	54.08	35.47
9 to 10	12.68	22.62	17.32	20.20	18.56	25.17	9.84
10 to 11	17.67	13.77	25.24	25.21	23.98	16.40	19.98
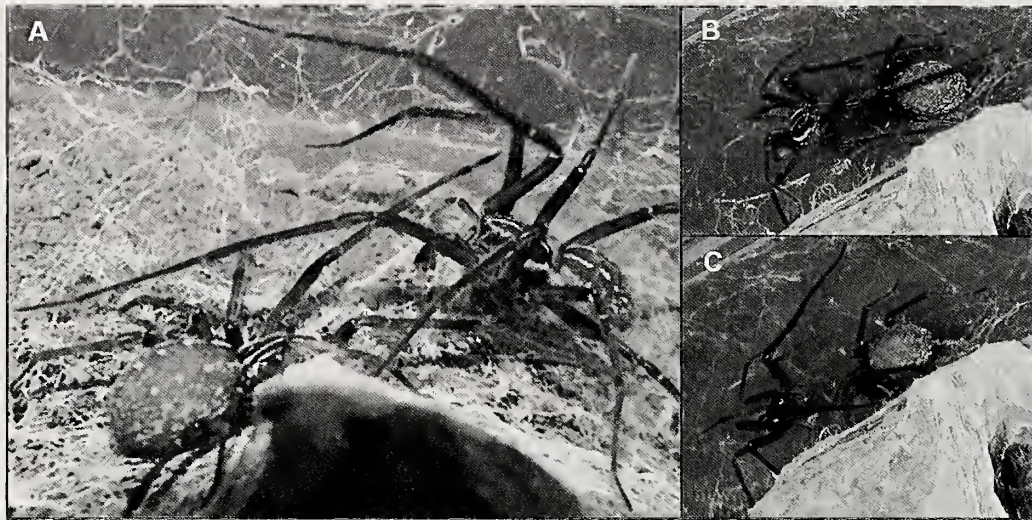
Figure 8A–C.—Courtship behaviors. A. Male strumming the web and stroking the female. B. Male stroking the female, female passive. C. Male stilting behavior.

beginnings of basic elements of adult webs were apparent with spiders forming small funnel retreats, followed by a very small sheet and tangle lines in the fourth instar (Fig. 6A, B). Retreats were either located in the middle or at one side of the web. Web complexity and size increased from one instar to the next, including multiple retreat entrances, sheet expansion and additional tangle lines (Fig. 6).

Cribellate silk did not appear in the web until instar 8. Nearly simultaneously, cribellate lines were observed in the retreat, along with an orb-like spiral in the sheet. This was followed in later instars with heavy or thick cribellate lines in the tangle that eventually covered the majority of the web in the eleventh or penultimate instar, giving the webs a fuzzy appearance. Of the three randomly selected spiders measured for morphological growth and web ontogeny, two were penultimate males and one penultimate female. These males actively maintained their webs and laid down cribellate lines.

The twelfth instar, or adult stage, saw changes in male web use. Males were observed or collected outside of webs or in female retreats in the field. Males in their lab containers laid down a circular sheet-like web composed of dragline silk. Females continued to lay down cribellate lines on their webs and tangle scaffolding until egg sac production. In the laboratory, most adults took refuge under the sheet, instead of maintaining a retreat. This was apparently due to the artifact of the short square containers used to house the spiders in the lab colony. Webs in the field (n > 100) had a much more vertical stratification and multidimensional structure compared to those in the lab.

Egg sac construction: Egg sacs in the field (n >10) were similar to those constructed in the lab (n > 20) (Fig. 3). Females constructed egg sacs in a stereotypical pattern (n > 20). First, they erected a hammock-like structure, with three to four attachments at the ends to the top and sides of the container (or retreat if in the field) (n > 10) (Fig. 3A). Next, they added silk to form a much thicker central disc at the center of the hammock, followed by a spherical bowl underneath this disc. All of this was done while hanging upside-down. They seemingly sealed the disc to the bowl, then

while hanging upside down, directly below the bowl, deposited eggs and fluids into the bowl structure. Afterwards, they laid silk over the entire bowl, reinforcing it (Fig. 3A), and then added cribellate silk lines that eventually covered the entire egg sac, completely covering it section by section. Lastly, females took pieces of the substrate in their chelicerae and placed them against the cribellum silk where they adhered (Fig. 3B), presumably serving as camouflage for the egg sac (Fig. 3B). Egg sacs were constructed singly or in pairs, about 1–2 weeks apart. Virgin females in the lab occasionally constructed egg sacs and deposit unfertilized eggs (n >10).

Courtship behavior.—Courtship began with the male orienting toward the female. Typically, in the lab, orientation by the male was preceded by preening (n = 20), during which time the male cleaned his palps and first two, or sometimes three, pairs of legs between his chelicerae. At this time, the majority of setae on the legs were fully visible and erect (see video S1, online at http://dx.doi.org/10.1636/JoAOS-15-004.S1). Once oriented toward the female, likely through vibratory cues, the male shook his abdomen while plucking the web with his first and second pairs of legs (Fig. 8A, see also video S2, online at http://dx.doi.org/10.1636/JoAOS-15-004.S2). If receptive (n = 16), the female generally responded by tapping the web with her legs I and sometimes legs II. If not receptive, the female lifted the web around her with all four pairs of legs and forcefully pushed the web downward, as though shaking out a rug. If the female tapped, the male paused, then approached her and strummed the web and stroked her carapace and abdomen with his first pair of legs, which are much longer than those of the female (Fig. 8B). He interrupted stroking for variable periods to shake his abdomen. Sometimes the female tapped or plucked in response, sometimes repeatedly. Eventually (n = 35), the male 'stilted' up, standing as tall as physically possible on all four pairs of legs, and shook his abdomen, typically above the female carapace (Fig. 8C, see also video S2, online at http://dx.doi.org/10.1636/JoAOS-15-004.S2). He did this stilting and shaking sequence up to three to four times. If she remained still, he deposited a 'bridal veil' of silk across the female's carapace and legs. This

was not a restraint, as the female could easily break the lines. If the female was receptive ($n = 16$), she exposed her epigynum by laying nearly completely on her side while the male silked the bridal veil (see video S3, online at http://dx.doi.org/10. 1636/JoA0S-15-004.S3). As the male continued to stroke her, he gathered her legs in towards her body with his long first pair of legs. The male then copulated, using his left palp to transfer sperm into the opening of the left spermatheca and vice versa. He did this while leaning across and over the female. Coupling lasted several seconds, and the male appeared to hook the female's epigynum with the RTA or median apophysis of his partially inflated palp. When the palp engaged, the hematodocha rapidly expanded once and then deflated, which took less than a second once the embolus was engaged (see video S4, online at http://dx.doi.org/10.1636/ JoA0S-15-004.S4). Typically, both parties immediately moved rapidly apart. In a few instances ($n = 3$), the female and male slowly separated a short distance and then resumed courtship, but the majority of interactions were characterized by a rapid, dramatic separation. On several occasions, the bridal veil sequence was repeated three or more times before successful copulation occurred. The female slowly broke the silk veil and the male would then re-approach her with web strumming and carapace stroking. There was a total of 35 documented interactions; 24 were initial pairings ($n = 16$ copulations with single spermatheca), 11 reintroductions or subsequent interactions ($n = 6$ copulations, 5 rejections). Two subsequent exposures led to multiple copulations ($n = 4$) with alternation between right and left sides each insertion. An extended courtship sequence which led to two copulations is presented in supplementary materials (see video S5, online at http://dx. doi.org/10.1636/JoA0S-15-004.S5).

Males of *T. perfuga* did not possess epiandrous spigots (Mallis, unpublished SEM data) and, therefore, may load the palps with sperm and seminal fluid deposited on the web. No observations were made of males constructing a sperm web or priming the palps. While courting, however, both male palps were partially expanded. The male did preen at times just after or between copulations if left in with the female, cleaning the palps and first, second and at times even the third pair of legs with his chelicerae. No stridulatory mechanisms, such as a file, were observed on the abdomen or carapace of adult male *T. perfuga* specimens.

In the initial 24 pairings, 16 females were receptive, 6 were not receptive, and 4 males did not court. Subsequent exposures ($n = 11$) of females (both mated and not) using the same ($n = 7$) or different males ($n = 4$) resulted in more successful courtships with some pairs ($n = 6$) copulating multiple times (up to four times before removal of the male or the female retreated or became non-receptive. Females with egg sacs ($n = 2$) were not receptive to courtship, and either ignored the male ($n = 1$) or non-aggressively drove them from the web ($n = 1$). All virginal females had "plugs" prior to courtship encounters and these plugs generally appeared soon after molting to adulthood. One female was examined under the dissecting scope, post-copulation, and had a plug on left side (non-insertion) and no plug on the right (successful copulation). However, within a day of copulation, a plug appeared on the right side. All mated females had sclerotized plugs with the same appearance as the epigynal plugs observed in virgin females.

DISCUSSION

Life history.—While *T. perfuga* took 9 – 12 molts to reach adulthood, smaller numbers can be seen in deinopoids, such as 6 – 7 instars to reach maturity after emergence in *Hyptiotes cavatus* (Hentz, 1847). Similar to *T. perfuga*, some *Pardosa* C.L. Koch, 1847 have multiple egg sacs, with at least 30+ eggs in each and follow an approximately two-year life cycle from egg sac emergence to reproduction and death (Buddle 2000). The purported sister species of *T. perfuga*, *T. radiata* reaches maturity in 9 instars (8 molts) for males and 10 instars (9 molts) for females. The time to reach adulthood was similar to that observed in the lab for *T. perfuga*: approximately 187 days for males and 229 days for females (Barrantes & Madrigal-Brenes 2008).

Growth.—Whereas the overall growth from second instar to adulthood was over 1000% for some structures, the amount of growth varied between different instars. Despite a small sample size and, therefore, a lack of statistical testing, there appears to be less relative growth or slower growth rate between instars 5 and 6 than any other stage. This warrants further study and may be due to more energy invested in developing structures such as the calamistrum (instars 5 and 6) and cribellum (instars 7 and 8) becoming functional (Table 1, Fig. 4) than to morphological growth. Interestingly, Barrantes & Madrigal-Brenes (2008) do not report any apparent slowing of growth between instars, particularly the seventh instar when cribellate silk first appears in the webs of *T. radiata*.

Allometric growth occurs between the penultimate and adult molts in leg I of males, as has also been observed in *T. radiata* (Barrantes & Madrigal-Brenes 2008). Similarly, in *Pisaurina mira* (Walckenaer, 1837), mature males have longer legs relative to the overall body size than adult females, particularly the first pair of legs (Anderson & Hebets 2016). Anderson and Hebets (2016) attribute this to allometry potentially driven by sexual selection. This is similarly hypothesized by Barrantes & Madrigal-Brenes (2008) for *T. radiata*, as in the field, males were collected on or near female webs, suggesting males abandon their webs in search of females, as we suspect for *T. perfuga*. They proposed that longer legs lead to larger step sizes to bridge the distances between male and female webs or to escape cannibalistic females; however, they did not associate the longer pair of legs with courtship behavior or explicitly with sexual selection as a possible mechanism for the allometry in Leg I (Barrantes & Madrigal-Brenes 2008).

Ontogeny of cribellum, calamistrum and cribellate silk use.—The combing behavior for production of cribellate silk lines is similar to that reported for the closely related *T. radiata* (Eberhard 1988). Individual spiders varied in their favored use of the right or left leg for combing (Mallis, pers. obs.). Some switched combing legs between one line and the next. Despite the physical presence of a cribellum and a partial to full calamistrum, cribellate silk does not appear until the eighth instar. This is interesting, as many zoropsid spiders have varied use of cribellate silk throughout their life cycles (Lehtinen 1967; Griswold et al. 2005). In the closely related *T. radiata*, the apparent non-functional status of the cribellum

and calamistrum in early instars is suggested by the lack of cribellate silk in the web until the seventh instar (Barrantes & Madrigal-Brenes 2008). This was speculated to be due to the energetic costs of producing cribellate silk, or a reemergence of a plesiomorphic condition (Barrantes & Madrigal-Brenes 2008). In *Hyptiotes cavatus*, newly emerged second instars also lacked a functional cribellum and calamistrum and did not form a web, but simply hung by a single line until molting to the third instar (Opell 1982).

Ontogenetic changes in web structure.—Silk played a role in many facets of life for *T. perfuga*, from foraging and shelter, to constructing egg sacs and in eourtship. Early instar webs had many characteristics of adult webs, but on a smaller, simpler scale and without cribellate silk (Fig. 6). These are acquired in the following order: basic retreats; small sheets; and knock-down lines. At the eighth instar, cribellate silk appeared in an orb-like spiral in the sheet, and subsequently throughout the web structure (Figs. 6C, D & 7). The laek of retreats in lab spiders was likely due to the artifact of being in the lab in a small container, as most field-eaught or observed spiders actively used retreats at all life stages (Mallis, pers. obs.). Male *T. perfuga* lose functionality of the cribellum in adulthood. Females line the sheets, edges of their web and tangle with cribellate silk, and maintain the webs until egg sac production. Cribellate silk is not only used for prey capture, but also likely plays a role in courtship, propagating male and female acoustic signaling (see Courtship below).

In the web ontogeny study of *T. radiata*, Barrantes & Madrigal-Brenes (2008) reported that in the field, second instar spiderlings did construct a collective molting web inside the mother's retreat and dispersed after molting to the third instar, as in *T. perfuga*. In the lab, second instars removed immediately after emergence from the egg sae did not readily spin a web individually (Barrantes & Madrigal-Brenes 2008). Third instar *T. radiata* constructed a dense horizontal sheet with retreats either below or above the sheet covered by tangle lines. Webs were expanded through subsequent instars. Most importantly, the seventh instar is when cribellate silk lines are observed in the tangle and sheet of the web, but the authors do not indicate in what pattern it was observed (Barrantes & Madrigal-Brenes 2008).

Similar to *T. perfuga*, the first capture webs of *Hyptiotes cavatus* in the form of a horizontal orb are constructed in the third instar, and subsequently become a cribellate triangular slice of an orb held tautly by the spider as the hub itself (Opell 1982). Males also ceased web production or maintenance in adulthood (Opell 1982). This has also been demonstrated in another uloborid, *Uloborus diversus* Marx, 1898, where second instar webs were horizontal orbs, without cribellate spiral silk (Eberhard 1977). Adult male *Uloborus* lack a functional cribellum and their web structure, if any, was similar to a second instar web (Eberhard 1977). In the ecribellate modified orb web araneoids, similar ontogenetic patterns are observed with early instars spinning vertical sticky orbs and adults using modified webs (Eberhard 1985, 1986). In the communal araneid, *Cyrtophora moluccensis* (Doleschall, 1857), second instar spiderlings formed a nursery web, similar to the collective molting web of *T. perfuga*, while adults had communal webs which consisted of individual orbs (Berry 1987).

Deinopoidea (cribellate horizontal orb-weavers) are more closely related to the RTA clade than to the Araneioidea (viscous silk orb-weavers, etc.), as previously thought, making the historical "Orbiculariae" paraphyletic (Bond et al. 2014; Fernandez et al. 2014; Garrison et al. 2016). The orb web is considered plesiomorphic for the deinopoid Uloboridae, and the modified cribellate webs of *Hyptiotes* Walckenaer, 1837 (triangular orb, spider as the hub) and *Miagrammopes* O.P.-Cambridge, 1869 (single capture thread) as derived or apomorphic traits (Opell 1982). Given these recent phylogenetic discoveries in the evolutionary history of spiders, one would expect to find remnants of orb-weaving behavior or silk use in members of the RTA clade (Agnarsson et al. 2013). *Tengella* spiders are members of the RTA clade, but, as demonstrated by the spiral pattern of cribellate silk, still exhibit some deinopoid orb-weaving behavior. This is consistent with the recent studies reporting Deinopoidea ancestor to the RTA clade, and the ecribellate "Orbiculariae" as sister to the Deinopoidea + RTA elade (Agnarsson et al. 2013; Bond et al. 2014; Fernandez et al. 2014; Garrison et al. 2016).

The initial orb-like spiral of cribellate silk only occurred across instar 8 (Fig. 7). Without a web ontogeny study, these behavioral and structural characters that reflect the deinopoid and orb web ancestry would have been missed. Using *T. perfuga* as a focal study system, it would be of particular interest to move from a web ontogeny study to a comparative study of silk spigot ontogeny across cribellate silk users including the former Orbiculariae and the RTA clade. A complete spigot ontogeny dataset of *T. perfuga* is forthcoming and a phylogenetic comparative analysis of spigot ontogeny data for several species is ongoing. These studies can further elucidate ancestral orbicularian traits, such as the cribellate spiral reported here in *Tengella*, in both cribellate and non-cribellate silk using spiders from the RTA clade.

Egg sac construction.—Camouflaging of the egg sac by the female and tolerance of second instars is recorded in numerous spider clades, such as the tetrablemmid *Monoblemma muchmorei* Shear, 1978 (Edwards & Edwards 2006). In *T. radiata*, both lab and field observations reported similar camouflage techniques and placement of the egg sac by the female in her retreat (Barrantes & Madrigal-Brenes 2008).

Courtship behavior.—*Tengella perfuga* has similar mating behaviors to those reported for *T. radiata* (Barrantes 2008), such as the male abdomen shaking and approach, as well as the strumming of the female web. Females, as in *T. perfuga*, assumed a passive position on their sides, exposing the epigynum to the male (Barrantes 2008). Similar broader descriptive phases could be identified as in Barrantes (2008). These include (1) male preening and orientation to female, (2) courtship performance, and (3) copulation. Whereas the basic steps tended to follow the same order, there were some notable differences. Female responses to the initial courtship of the male involved plucking or strumming with legs I and II, whereas *T. radiata* females used their palps (Barrantes 2008). *Tengella radiata* males had a rocking behavior while stilting (Barrantes 2008), whereas *T. perfuga* males remained still and, rather than rocking, either shook their abdomen or strummed the web. The process of male courting, female strumming response, and male advancement towards the female described

for *T. radiata* by Barrantes (2008) is similar to that observed here in *T. perfuga*. Whereas Barrantes (2008) observed female attack behavior to repel male suitors, that kind of aggression was not observed in the *T. perfuga* females in the lab. If a lunge had occurred, no contact was made with the male and typically legs I and chelicerae were not outstretched as though attacking (Mallis, pers. obs.). The male position during copulation was different than that reported for *T. radiata*, where male and female ventral surfaces are positioned parallel to each other and touching while faeing in opposite directions (Figs. 3 & 4 in Barrantes 2008). *Tengella perfuga* males instead reach across and over the female dorsum to access the epigynum typically while faeing nearly perpendicularly with the female. At times, due to web constraints, they were positioned parallel to each other. Palpal insertion and "flubs," defined by Barrantes (2008) as rapid scraping motions of the palp or failed embolus insertion attempts in *T. radiata*, were similar in *T. perfuga*. For example, once the palp successfully engaged, the hematodocha expanded once, lasting less than one second (Barrantes 2008). Also as in *T. radiata*, if multiple successful copulations were allowed to proceed, *T. perfuga* females would expose the alternating side for copulation (Barrantes 2008). Sometimes female *T. perfuga* did not accept further copulation attempts and males were removed from the containers.

The most notable and obvious difference between these two closely related species was the lack of a bridal veil in *T. radiata*, as Barrantes (2008) never made mention of this in his courtship study. It may be that males did employ this behavior; it was just not reported in the two courtship encounters of the study. Aneedotally, the first author collected *T. radiata* in Nicaragua (2012) for similar purposes, but was unable to get a viable colony established. She introduced a male *T. perfuga* to a female *T. radiata* who accepted his courtship advances, including a heavy bridal veil, and allowed him to copulate. Although an egg sac was produced, it was non-viable (Mallis, pers. obs.). While female *T. radiata* were not reported to end courtship and/or copulation by breaking out of the bridal veil as *T. perfuga*, the movements of the legs to pull themselves back to standing on the sheet as reported by Barrantes (2008), were similar to those of breaking out of the veil to stand in *T. perfuga*.

Similar overall mating behaviors have been observed not only in *T. radiata*, which was selected for study because it is a cribellate relative of the Lyeosoidea, but also in closely related lyeosoids and agelenoids (Stratton et al. 1996; Huber 1998; Barrantes 2008). While courting a female, *Pisaurina mira* males used their legs to help wrap her with silk before and during copulation (Anderson & Hebets 2016). Consequently, males typically with longer forelegs (Leg I), who could wrap females, had increased sperm transfer and a lower likelihood of falling prey to cannibalism, suggesting some form of sexual selection occurred (Anderson & Hebets 2016). Many araneid males also employed a plucking or strumming of the web behavior (i.e., Berry 1987).

While further study is needed, the potential lack of choosiness on the part of females was not very surprising because in the field, males must wander to find females and encounter rates could be inherently low at some field sites. Also, several virginal females, collected in the field or reared in

the lab, had epigynal plugs prior to mating (Mallis, pers. obs.). While this has never been reported before in a spider, it is not entirely surprising, as some studies, particularly with *Leucauge* White, 1841 spiders, have found that females participate in producing a mating plug both during and after copulation (Aisenberg & Barrantes 2011). Therefore, it would not be a stretch to consider females producing an epigynal plug prior to eopulation, which is a very novel observation. It is possible that the consistent behavior of flubs or 'scraping' by the male palp prior to insertion was an effort to remove the plug and that played a role in female choice. Given their relative ease of rearing and large size, combined with variable mating behaviors and other attributes, spiders of the genus *Tengella* lend themselves well as a model system for both sexual and silk use evolution in spiders.

ACKNOWLEDGMENTS

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Web building and prey wrapping behavior of *Aglaoctenus castaneus* (Araneae: Lycosidae: Sosippinae)

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Abstract.   Funnel webs are common and widespread taxonomically, but little is known about how they are built or details of their structure. *Aglaoctenus castaneus* (Mello-Leitão, 1942) (Lycosidae) builds horizontal, densely meshed funnel webs of non-adhesive silk, with a tangle of lines above. Web construction behavior was unique in that the spider frequently laid swaths of lines rather than simple drag lines, both to float bands of fine lines on the breeze as bridges to distant objects and to fill in the sheet. Spiders utilized special spinneret movements to widen the swaths of lines that they laid on sheets. These movements have not been seen in web construction by other araneomorphs, but are were similar to and perhaps evolutionarily derived from those used during prey wrapping by many other species. Observations, made with a compound microscope, of the construction behavior of the agelenid *Melpomene* sp. O.P. Cambridge 1898, and of lines and attachments in sheets of these species and another funnel web spider, the zoropsid *Tengella radiata* (Kulczyński, 1909) demonstrated the possibly general nature of including obstacles in the web. This probably disadvantageous behavior may be related to constraints in selecting web sites imposed by the need for sheltered retreats, or to the spider's inability to remove preliminary lines. The observation also showed the importance of further improvements in the discriminations made between "sheet" and "brushed" webs in recent discussions of spider web evolution.

Keywords: Sheet web, funnel web, web evolution

The evolutionary history of prey capture webs in spiders includes complex series of acquisitions and losses (e.g., Kaston 1964; Kullmann 1972; Vollrath & Selden 2007; Bond et al. 2014). "Sheet" webs constitute one widespread class of webs with a more or less planar, horizontal dense array of lines where the spider walks and captures prey. One common and widely distributed type of sheet web built by taxonomically diverse spiders is the "funnel web", a tightly meshed, approximately horizontal sheet of generally (though not always) non-adhesive lines on top of which the spider walks, and which is connected at one edge with a tubular retreat. Some funnel webs also have a tangle of lines above the sheet. Funnel webs occur in Dipluridae (Coyle 1986; Paz 1988; Viera et al. 2007; Eberhard & Hazzi 2012), Agelenidae (Bristowe 1958), Zoropsidae (Eberhard et al. 1993), Pisauridae (Nentwig 1985; Santos 2007) and Lycosidae (Hingston 1920; Brady 1962; González et al. 2015). Funnel web construction has almost never been observed directly (see Rojas 2011; González et al. 2015). Nevertheless, dense swaths of fine lines (presumably from the aciniform glands) are said to be laid across other lines in the sheet and seldom (if ever) attached to them with piriform attachment discs in some "brushed" funnel webs (Blackledge et al. 2009). Funnel webs have probably evolved convergently in different groups, but details have yet to be resolved.

Most species in the large, monophyletic wolf spider family Lycosidae (approximately 2400 species) (Platnick 2016) are vagrant hunters and do not build any silk structure to capture prey. However, a few genera build funnel webs and recent molecular studies suggest that lycosids may be descended from web builders (e.g., Bond et al. 2014; Fernández et al. 2014). These species, which tend to have longer posterior lateral (PL) spinnerets (Yoo & Framenau 2006), are in the genera *Hippasa* Simon, 1885, *Aulonia* C.L. Koch, 1847, *Anomalosa* Roewer, 1960, *Venonia* Thorell, 1894 (the latter two are thought to be sister genera – Yoo & Framenau 2006), *Sosippus* Simon, 1888 and *Aglaoctenus* Tullgren, 1905 (the only two genera of the New World subfamily Sosippinae) (Hingston 1920; Brady 1962; Santos & Brescovit 2001; Viera et al. 2007; González et al. 2015). The funnel webs described for sosippine lycosids resemble those of Agelenidae in several respects, and fit the characterization of "brushed" sheet webs (J. A. Coddington, pers. com.): they consist of dense, extensive, approximately horizontal sheets that are connected at one edge with a tubular retreat, and often have a tangle of lines above the sheet (Santos & Brescovit 2001; Viera et al. 2007; González et al. 2015).

Both morphological and molecular traits indicate that Sosippinae is monophyletic (Santos & Brescovit 2001; Murphy et al. 2006). Conclusions from these recent analyses were contradictory, however, regarding the evolution of web construction in lycosids. One model consistently suggested a non-funnel web ancestry for the family Lycosidae, and four independent origins of funnel webs within Lycosidae. A second suggested that funnel web construction was an ancestral lycosid trait (Murphy et al. 2006), in accord with the speculation of Jocque & Alderweireld (2005) that lycosids were originally forest-dwelling web builders.

With respect to sossipine web structure, all four species in the genus *Sosippus* for which observations are available build funnel webs (Brady 1962), but with somewhat different designs. The tunnel retreats of *S. californicus* Simon, 1898 and *S. texanus* Brady, 1962 were often in cavities in the ground (Brady 1962), while those of a species in Costa Rica (presumably *S. agalenoides* Banks, 1909, the only species reported from this country) were often a meter or more above the ground, and the silk tunnels were built among the leaves and branches of weeds and shrubs (W. Eberhard, unpub. obs.). The sheet portions of the webs of *S. agalenoides* and *S. californicus* were relatively larger than those of *S. texanus* and

S. floridanus Simon, 1898 (Brady 1962). The closely related, widely distributed South American sosippine genus *Aglaoctenus* includes five species (Santos & Brescovit 2001; Piacentini 2011). Santos & Brescovit (2001) characterized *Aglaoctenus* webs as "a horizontal non-adhesive sheet with width and length between 4.5 and 90 cm" connected to a tubular retreat, with "vertical barrier threads" above the sheet. Photos of the webs of both *A. castaneus* (Mello-Leitão, 1942) (Santos & Brescovit 2001) and *A. lagotis* (Holmberg, 1876) (Viera et al. 2007; González et al. 2015) conform in to this description. However, the limited descriptions of *A. yacytata* Piacentini, 2011 webs (of a mature female with eggs, and a juvenile), mention a silk tube but no sheet (Piacentini 2011).

Funnel web construction behavior is surprisingly poorly known, despite the wide taxonomic and geographic range of groups that build funnel webs and their relative abundance. Two brief studies of funnel web construction were performed in captivity. Rojas (2011) studied the early stages of web construction of the agelenid *Melpomene* sp. and González et al. (2015) observed the sosippine lycosid *A. lagotis*. One brief field study (Eberhard & Hazzi 2012) described the early stages of web construction by the diplurid *Linothele macrothelifera* Strand, 1908. All three studies were fragmentary in many respects. The diplurid's behavior was the simplest. The spider appeared to lay only one kind of silk, which consisted of a swath of lines produced while the spider moved about on the substrate near the tubular retreat; there was no preliminary skeleton of lines. On the other hand, the two araneomorph species performed two types of building behavior very early in web construction. One consisted of more or less straight, approximately radially oriented movements from near the mouth of the tunnel to the edge of the web and beyond to attach lines to the substrate and to each other, thereby producing a support or "skeleton" sheet. The second type consisted of more erratic, wandering movements across the skeleton web, during which the spider swung her abdomen repeatedly from side to side, often with her PL spinnerets spread laterally, filling in the skeleton with a sheet of fine lines. The two types of behavior alternated, and their relative durations varied. These two species also differed from the diplurid in attaching lines to each other and to the substrate with brief dabs of the tip of the abdomen that probably produced piriform attachment discs (mygalomorphs lack piriform glands). Fragmentary observations (Hingston 1920) indicated that the lycosid *Hippasa olivacea* (Thorell, 1887) also built a skeleton web of single lines, and then filled it in with swaths of finer lines. By selectively sealing individual spinnerets of *A. lagotis* with paraffin and observing close-up video recordings of spiders constructing webs, González et al. (2015) deduced that the support or skeleton lines emerged from the anterior lateral (AL) spinnerets (and may thus be major ampullate gland lines), while the fill-in lines came from the PL spinnerets (and are thus aciniform gland lines – see below). They also reported on the likely metabolic costs of web construction, in terms of reductions in the spider's immune responses.

The observations of Santos & Brescovit (2001) concerning the approximate numbers and locations of the spigots of different glands on different spinnerets in *Aglaotenus* provide an important morphological foundation for observations of spinning behavior reported here. The AL spinneret has two major ampullate gland spigots and 70-80 piriform spigots; the posterior median (PM) spinneret has 30-40 aciniform spigots; and the longer PL spinneret has 30 aciniform spigots, all on the medial-ventral surface of the elongate triangular distal segment. We thus assumed in the descriptions below that lines that emerged from the PL spinnerets were aciniform lines.

Here we report observations of *A. castaneus* webs, construction behavior and prey-wrapping, and observations of the webs and behavior of *Melpomene* sp. (Agelenidae) and *Tengella radiata* (Kulczyński, 1909) (Zoropsidae). This report has several objectives: to document some basic differences between funnel web construction behavior and the better-studied orb web construction that are important for understanding the evolution of funnel web designs; to provide a basis for future comparative studies of funnel web construction; to point out the potential usefulness of several behavioral details as taxonomic characters (see Eberhard 1982, Kuntner et al. 2008 for examples in other spiders); to document the possible evolutionary origin of one aspect of funnel web construction in prey wrapping behavior; and to provide criteria to help improve some over-simplifications made in recent discussions of the evolution of spider webs.

METHODS

Study area.—We observed *A. castaneus* in the field on 21–24 August, 2013 in riparian and secondary dry forest in the Parque Natural Regional El Vínculo (3°, 50', 23" N, 76°, 18', 07" W; elev. 950–1100 m), a tropical dry forest zone in the Holdridgian classification (Espinal & Montenegro 1963). The reserve is contiguous with the edge of the floor of the Cauca Valley, 3 km south of the Municipio de Buga, Valle del Cauca, Colombia (elev. 1020 m).

Field observations.—The spiders were extremely abundant (Cabra-García et al. 2010). We measured the approximate width and length of each web with a ruler and sketched the outline of the sheet. In order to observe web construction and freshly-built webs in which patterns in lines could be more easily deciphered, we removed webs in the field in the afternoon, leaving only the mouth of the tunnel and the tunnel itself intact (the mouth of the tunnel will be termed the "retreat" hereafter). We then waited in the evening to watch spiders build (until about 23:00), and returned for further observations at 04:00 the following morning. We repeated this procedure with different webs on three days. We made recordings of a total of >60 min of the behavior of two mature females using a SONY HDR-HC9 camera equipped with and near infra-red illumination ("night shot") and a +4 closeup lens; the spider's body could fill the frame in closeup shots.

This recording method of illumination provided an unanticipated payoff. Occasionally, when the angles of view were favorable, the illumination glinted off lines that the spider was producing; we used these occasional glints to determine the positions of lines. This technique had the limitation that only those lines that were at favorable angles to the illumination were visible. In some cases, different lines "lit up" in successive frames of a recording, demonstrating that not all the lines that a spider was producing at a given moment were necessarily visible in a given frame. There were often many successive

frames in which no lines were visible, followed by one or a few frames in which many lines were visible. Given our substantial recording time, however, we were able to build up general ideas regarding some common spinning processes. Our drawings represent particular spiders at particular instants, but these moments were chosen to illustrate what were typical aspects of behavior. Similarly, our ability to see lines only occasionally and perhaps incompletely meant that we had to assume that an "attachment" occurred each time the spider brought her spinnerets into contact with other lines or a solid substrate (these presumed attachments will be called "attachments" hereafter), and that attachments involved the production of piriform discs. We were only able to confirm directly that some attachments had occurred by observing that lines adhered to previous putative attachment sites (Fig. 6a); in no case did we check for attachment discs. When the spider swung her abdomen laterally, we termed the side toward which the abdomen moved as the "leading side", and the other as the "trailing side". We also photographed 35 webs after dusting them with talcum powder. We left six powdered new webs built on the first night intact, and checked them for further additions on the following two days.

We collected samples of lines in the sheets of newly spun webs or of large repair sectors that had been built the night before for *A. castaneus, Melpomene* sp., and *T. radiata*; we pressed a plastic ring (made from the top 2–3 cm of a plastic drinking cup) against the underside of the sheet, taped the sheet to the sides of this ring, and cut it free with scissors. We took care not to include the tangle above the sheet (this control was especially strict in *T. radiata* and *Melpomene* sp.). Sections of these samples were later carefully taped to microscope slides, cut free from the rings, and viewed without a coverslip or any further treatment at ambient indoor temperature and humidity in San Jose, Costa Rica. Although the sections of the sheets survived these treatments intact, it was possible that the tensions on lines on the slide were not the same as they had been in intact webs.

Observations of *A. castaneus* in captivity.—We observed web construction by four mature females and one penultimate male in two 30 × 20 cm terraria with the bottom covered with moist earth in which a retreat had been made by inserting a finger into the earth. We observed fragments of construction behavior of all four females, and filmed two of them. A black cloth was placed behind the cage to increase the visibility of the silk lines. Repair behavior was elicited by cutting a circular hole in the sheets of three females; one repair was filmed.

We tested for prey wrapping behavior using acridid grasshoppers, noctuid moths, and calliphorid flies. Because spiders only wrapped the flies, we then staged six wrapping episodes with flies, two for each female. Attacks were staged between 19:00 and 20:30, and filmed with a SONY HD-ACHD video camera. We also observed prey capture and wrapping with three gryllid crickets during the day in the field. One mature female *Melpomene sp.* was filmed in captivity during a bout of construction behavior that followed the capture of a fly on her relatively intact sheet web.

In order to avoid the use of the less specific "it" and thus increase the clarity of behavioral descriptions, we adopted the convenient illusion (as in Spanish, French, and German) that all spiders are females, and will refer to "her legs", etc. Because

of the problems in providing precise descriptions of web forms that do not correspond to common English words, and because of the history of inconsistent use of terms such as "sheet" and "funnel" in previous publications (see the discussions of Viera et al. 2007 and Blackledge et al. 2009), we use photographs and drawings extensively. We describe spinneret movements in some detail because they are useful in resolving which lines in the web resulted from which glands (González et al. 2015). Because of our reliance on glints to visualize lines, our descriptions may constitute only a partial list of the basic behavioral "vocabulary" of these spiders. In our descriptions we use the words "thick" and "thin" only with reference to the overall diameters of lines, and not to the diameters of the fibers that may or may not have comprised them. We use the category "orbicularians" (which may be paraphyletic – see Garrison et al. 2016) in only a descriptive sense, to denote araneoid and deinopoid families.

Specimens of *A. castaneus* were kindly identified by Adalberto Santos, and vouchers are deposited in the Museo de Entomología of the Universidad del Valle (MUSENUV), Cali, Colombia, and in lot "*A. castaneus* UFMG 4990" of the Universidade Federal de Minas Gerais, Brazil. Specimens of *Melpomene* sp., collected previously as part of the study of Rojas (2011), were identified by D. Ubick; specimens of *T. radiata* were also identified previously, as part of the study of Eberhard et al. 1993. Vouchers of *Melpomene sp.* and *T. radiata* are in the Museo de Zoología of the Escuela de Biología of the Universidad de Costa Rica.

RESULTS

***Aglaoctenus castaneus*: Webs in the field.**—The >100 webs that we observed in the field resembled in general terms the previous photographs and descriptions of the webs of *A. lagotis* and *A. castaneus* (Santos & Brescovit 2001; Viera et al. 2007; González et al. 2015). Each web consisted of an approximately horizontal, densely meshed sheet that was continuous with the lower surface of a tubular retreat at one edge; most webs also had a tangle of lines above the sheet (Fig. 1). We can add several details. The spider usually rested immobile day and night at the retreat on the upper surface of the sheet, fleeing briefly into the tunnel when disturbed, only to re-emerge a few minutes later. The tunnel's diameter was consistently very large compared with the size of the spider (Fig. 1b, c). The sheets of mature adults and penultimate nymphs were near but always somewhat above the ground (usually about 10–30 cm), rather than being on its surface (Figs. 1a, b, 2a–c); a few were much higher, up to >1 m above the ground. The outer portions of a few sheets had long straight lines or very sparse sheets just below them (Fig. 2a–c). Finally, five partially destroyed, detritus-laden webs were found in which there was a female carrying an egg sac.

When we removed approximately 20 sheets of adults and penultimate nymphs in the field, we found that more than half had at least fragments of a second, evidently older sheet with abundant holes and detritus a few cm beneath it, often draped loosely on the ground or other supports. The lax nature of the older sheets and their separation from the newer sheets suggested that the old webs had been at least partially destroyed by the spider before the newer sheets were built, and that spiders did not simply build a new sheet directly on
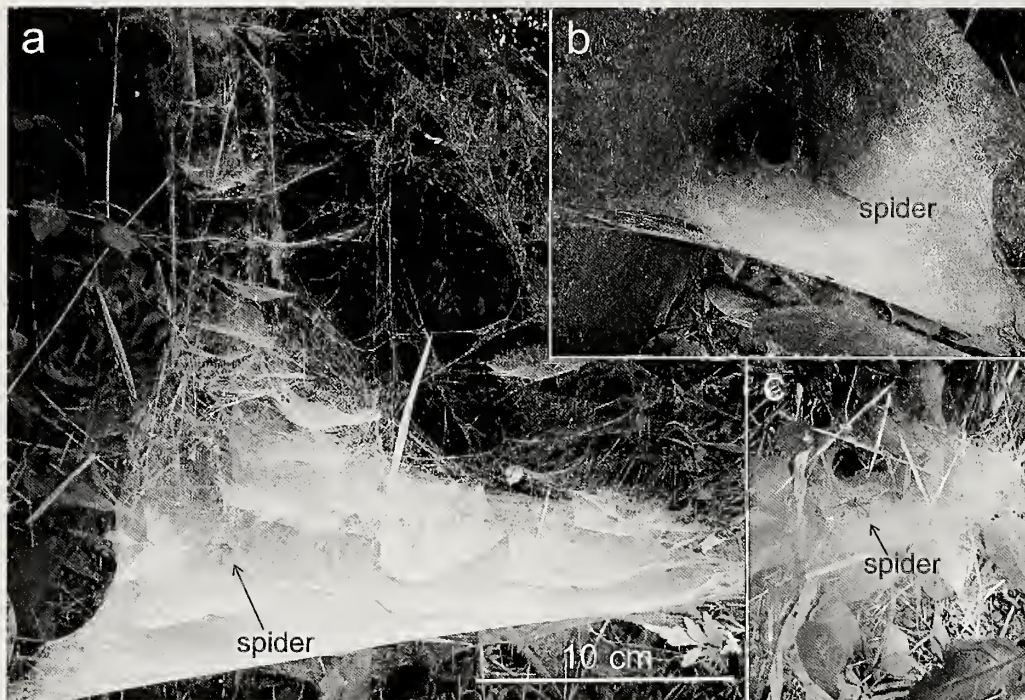
Figure 1.—Webs of *Aglaoctenus castaneus*. (a) Lateral view of the web of a mature female of *A. castaneus* with the spider (arrow) resting near the tunnel mouth on a typical, approximately horizontal sheet that was near but nevertheless above the ground. The tangle above this sheet (outlined in part by the large number (>18) of orb webs of the commensal uloborid *Philoponella* sp.) was especially high (95 cm). (b) Approximately horizontal web of a mature female at the base of a tree trunk; there were no supports available above the sheet, and the web had no tangle. The left edge of the sheet was more sparsely filled in than the rest. The spider (arrow) rested during the day in a typical position near the tunnel mouth, which was very large compared with the spider. (c) Web of a mature male, visible resting on the sheet near the retreat. This web differed from others in lacking multiple tiers.

top of a pre-existing sheet (though this did occur in captivity). We did not observe *A. castaneus* cutting silk lines, but it is worth noting the behavior used to cut silk lines in *A. lagotis* (M. González, pers. eomm.) differed from the cutting behavior of araneoid and deinopoid spiders. The lycosid appeared to break lines mechanically, rather than chemically, by tugging and pulling on lines with her chelicerae and her entire body.

There was usually a tangle of lines above the sheet. In adult webs, the tangle often extended 30-50 cm above the sheet, and sometimes up to >2 m; tangles often had multiple orbs (up to about 20) of adults and nymphs of the apparently commensal uloborid *Philoponella* Mello-Leitão, 1917 sp. (Fig. 1a). When coated with white powder, many of the tangle lines appeared to be relatively thick; but fine, lax lines were also abundant in some tangles. Both fine and thicker lines occurred in the tangles of newly built webs, and in webs that lacked commensals, so they were likely produced by *A. castaneus*. At some sites, where there were no attaehment sites available directly above the sheet, there was no tangle (Fig. 1b).

Some isolated attachment points for both the sheet and the tangle were very likely inaecessible from the retreat via walking (e.g., Fig. 1a), indicating that spiders likely used airborne bridge lines to obtain access to some web supports.

Detailed examination of lines near the edges of newly built sheets, where the densities of lines were lower and different types of lines were easier to distinguish, revealed both long, sparse, relatively straight and apparently thicker lines that were attaehed to supporting objects, and abundant apparently thinner lines that were often somewhat parallel or that

radiated from apparent points of attachment to the thieker lines (Figs. 2b, 3c, d). These two types of line probably correspond to the skeleton and fill-in lines ("DTT" and "DDT" lines) that González et al. (2015) observed in *A. lagotis* (the thicker lines may have been eables of fine lines, however; see descriptions of behavior below).

Another apparently new detail was that sheets almost always (except perhaps for that of one mature male—see below) had several small swaths of approximately 10–20 more or less parallel, thin, lax lines that were one to a few mm above the sheet (Fig. 2a) (we will call these "tiers"). We did not discern a pattern in the locations of tiers on the sheet, other than that they seemed to span small concavities. Often the swath of lines in a tier was up to one cm wide in places, and the fine lines often converged to at least some extent at one edge (arrow in Fig. 2b). Although the PL spinnerets were relatively long (about 1.0 mm in an adult female) and could be spread so their tips were about 1.75 mm apart, some tiers were thus often substantially wider (Figs. 2b, 3c, d). Some sheets also had larger, sparse sheets above the main sheet (Fig. 4).

In a few webs, one or more dead twigs or stems protruded through the sheet (Fig. 5a, b). There were also upward bulges in some sheets (Fig. 5c) where objects such as dead leaves just below the sheet projected upward.

Construction behavior.—*General movements:* We observed sheet but not tangle construction behavior. Ten of the eleven spiders whose webs were destroyed in the afternoon and then rechecked made a replacement web on the following night. In captivity, we observed two episodes of building by two spiders
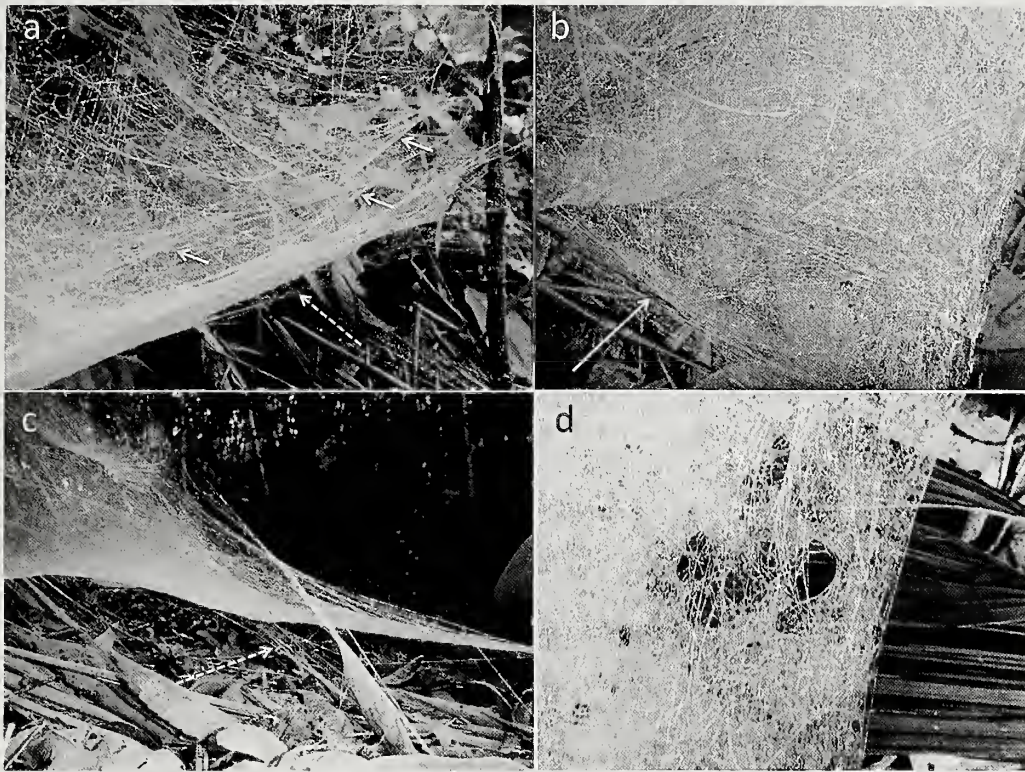
Figure 2.—Webs of *Aglaoctenus castaneus*. (a) Edge-on view of the far edge of a sheet. There is a complex array of small, sparse sheets ("tiers") of more or less parallel fine lines (solid arrows), and a possible "false start" just below the main sheet (dashed arrow). (b) Dorsal view of the far edge of the sheet. A clearly distinguished swath of widely diverging lines is attached at the edge of the sheet (arrow). (c) A larger "false start" group of lines below the outer portion of another sheet dashed arrow). (d) Holes produced in a newly built sheet where a cricket was captured.

in detail, from beginning to end. Construction behavior in these cases was intermittent, with bursts of activity that lasted on average 19 ± 7 min ($n = 26$), interspersed with pauses near the retreat.

We were not able to distinguish thick lines from thinner lines as they were being built; all glimpses of lines provided by glints revealed swaths rather than only one or two lines being produced, even during the early stage when long, apparently strong lines from the retreat were built (Fig. 6n); in no case was it certain that only thick lines were being produced. We suspect that many of the thicker lines in finished webs (e.g., arrow heads in Fig. 3b) consisted of cables of smaller diameter lines (perhaps including both fine aciniform gland lines and thicker ampullate gland lines).

Although spiders may have tended to lay thicker lines earlier in construction, thick lines and swaths of fine lines were often produced as part of the same process, even very early in construction (Fig. 6). In one web, the spider repeatedly ($n = 6$) laid long lines between the retreat (or lines nearby) to objects that were five or more body lengths away, where the far edge of the sheet would be. Typically, she did not attach the lines she was producing to any other lines on the trip away from the retreat, and walked on the vegetation at the far edge of the web before finally attaching one or a few times to a leaf or a twig (Fig. 6k); she then returned more or less directly to the near vicinity of the retreat apparently along the line she had just laid, again making few or no attachments along the way (Fig. 6c, d). Presumably these long lines corresponded to the

long, thick lines visible at the far edges of some finished webs in the field (Fig. 3c, d).

Close-up video recordings showed that even when the spider was laying such long lines, she produced swaths of multiple lines (Fig. 6d, n, o). Some of the fine lines that were laid along with the early skeleton web lines contacted other lines in the sheet, but others billowed loose in the gentle breezes, and may have been used to float bridge lines to distant objects. The maximum distance we saw a line float was 1 m. Construction of strong support lines also sometimes occurred later, during periods when the spider was apparently filling in the spaces between thick lines with swaths of fine lines. In sum, we were unable to distinguish stages of skeleton web construction and filling-in behavior (*sensu* González et al. 2015) in terms of thick and thin lines.

We did not see enough behavior to be able to be sure whether there were any patterns in the sequences of areas of the web in which the spider worked during early sheet construction, except that she frequently returned to the mouth of the tunnel. Occasionally she paused there for a minute or more, or immediately left again to continue construction in either the same or a different sector.

Details of spinneret and leg movements during early stages of web construction: Early in web construction (Fig. 6a, d, f), the two PL spinnerets were often spread laterally, with each emitting a swath of fine lines (Fig. 6f). Occasional lateral views suggested that these spinnerets were also flexed dorsally, and that the AL spinnerets were flexed ventrally (Fig. 6d). In some cases it appeared that, when one or both PL spinnerets were
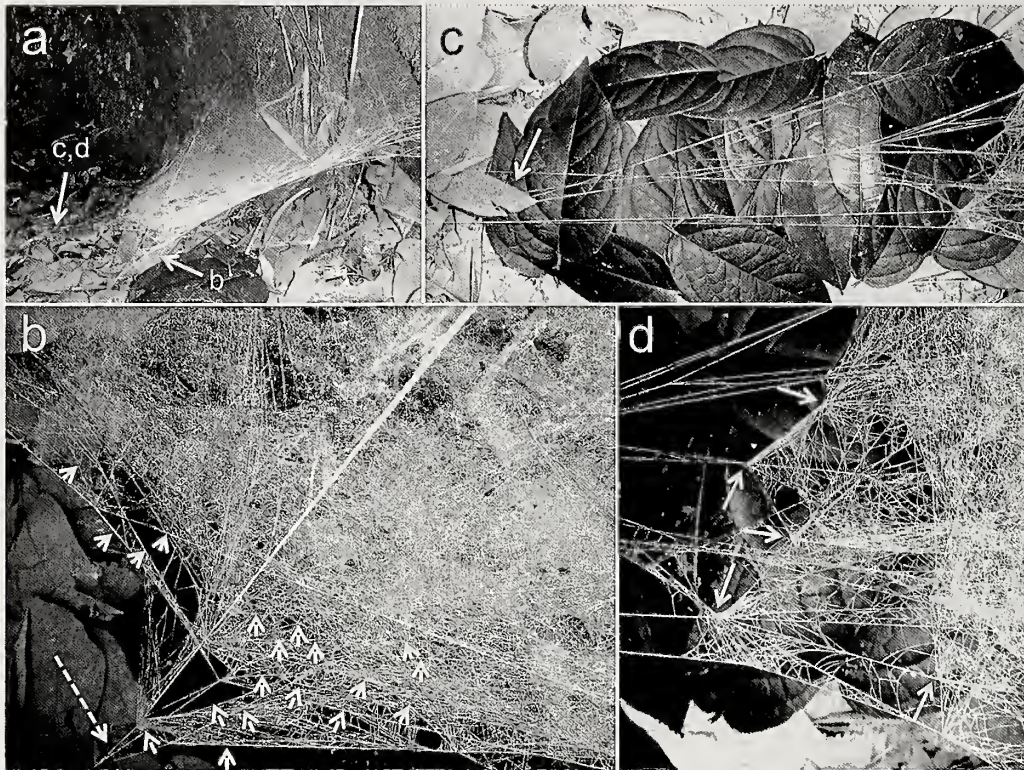
Figure 3.— Details of the far edge of a sheet, where the lines were less dense and thus more easily resolved, reveal an organization into skeleton and fill in lines. (a) View of the sheet built with its retreat against a buttress root near the ground, and almost completely lacking tangle lines. The two portions of this web illustrated in close-ups in (b)–(d) are indicated with arrows. (b) Some of the long, straight, and in at least some cases, perceptibly thicker "skeleton" lines near the edge are marked with arrowheads; (c) Several long "skeleton" anchor lines extended beyond the sheet. (d) Some of these skeleton lines shown in (c) were connected by apparently thin, loose (curved) fill-in lines; in some places, many apparent fill-in lines radiated from an attachment point (marked with arrows).

directed more posteriorly, the swath of lines condensed into what appeared to be a single line. A further complication was that the spider sometimes clapped or rubbed her PL spinnerets together repeatedly while she was walking, perhaps causing aciniform gland lines to adhere either to each other or to ampullate gland lines. In other cases, the PL spinnerets waggled medially rapidly but did not touch each other (left

Figure 4.—Webs of *A. castaneus* that had a sparse, "extra" sheet above the main sheet. (a) In this lateral view looking toward the tunnel mouth of a newly built web that nearly entirely lacked a tangle above, there was a sparse sheet of thin lines just above the main sheet (arrow). There was also a sector near the right edge of the sheet that was perhaps older, or less densely filled in. (b) Seen in lateral view, another web shows a more extensive tangle above, in which two "extra" sheets are visible (arrows). The right edge of the main sheet is relatively sparsely filled.

portion of Fig. 6b), or one waggled medially while the other was quiet; the consequences of these movements for the lines being laid were not clear.

The way the spider walked along a single long line (as, for instance, when she returned toward the retreat along a long line that she had just laid early in construction) was often strikingly different from that of orb-weavers, and indeed from any other published description of spider behavior that we know of. Instead of using the legs on both sides of her body to grasp the line and support her weight, the spider used mostly or exclusively the legs on only one side; the legs on the opposite side of her body were extended, and were either immobile or waved in the air below her body (Fig. 6c) (in some cases lower leg I was also used to grasp and contact the line along which she was moving). In one case, the spider began a return trip along a long line using the legs on both sides for three to four body lengths, and then shifted to holding the line with only ipsilateral legs (those on only one side of her body). In walking along the line, upper leg II (LII in Fig. 6e) followed upper I (LI in Fig. 6c), upper III followed upper II, and upper IV followed upper III (similar following behavior between adjacent ipsilateral legs is widespread in orb-weavers— Hingston 1922; Eberhard 1987a). In contrast, when *A. castaneus* walked on a solid surface such as a trunk, following behavior was uncommon; even though some movements were coordinated (e.g., III did not make its next step forward until
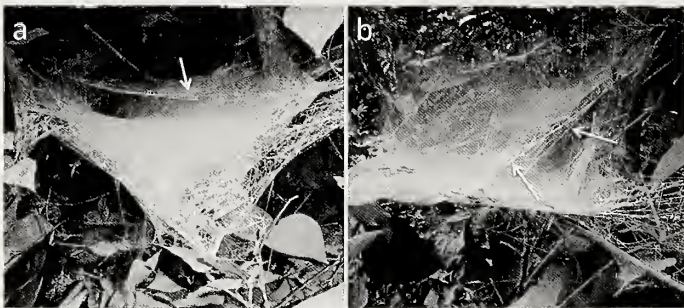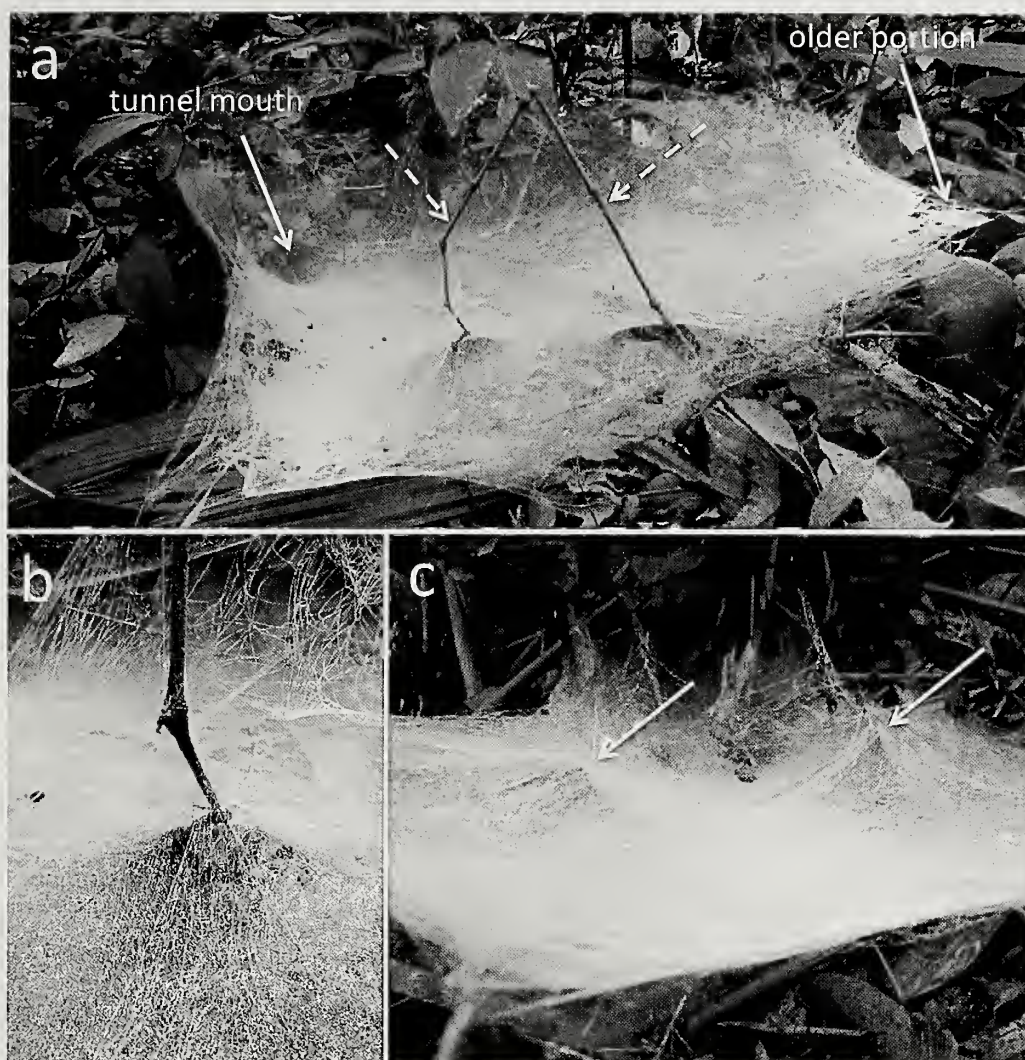
Figure 5.—Possible errors in planning. (a) This sheet had two stems (dashed arrows) protruding through it. Some sectors near the far left and the far right edge were apparently older, and had numerous small holes. (b) The lines attached to these stems sloped upward. (c) The "lumps" (arrows) in another sheet web were caused by dead leaves that projected into the plane of the sheet. The presence of obstacles such as these stems and lumps seems likely to slow the attacks of spiders; they are thus probably disadvantageous, especially in view of the webs' apparent poor abilities to retain prey.

just after ipsilateral IV had landed on the trunk), IV usually contacted the trunk far from III.

We noted two additional differences with orb-weavers and their relatives. There was never any indication that spiders broke and reeled up lines along which they walked. Nor did we ever see one leg IV holding the drag line as the spider walked and allowing it to slip through her tarsal claws (Eberhard 1982; Eberhard & Barrantes 2015). Occasionally, however, one leg IV rested on her drag line (and may have held it in some cases) at the moment it was being attached to the substrate after a long trip away from the retreat (Fig. 6e); the leg remained on the line while the spider turned and then grasped the line with her ipsilateral leg II (e.g., leg II followed ipsilateral leg IV) as she began to move back toward the retreat. On some other occasions, however, leg IV clearly did not contact the line as it was being attached when the spider attached to the substrate and then turned back toward the retreat (Fig. 6h). Holding the drag line with one leg IV was

more common when attaching to the substrate (5 of 12 cases in which this detail was clear) than when attaching to other silk lines (0 of 45 cases). It was not certain whether the ventral surface of tarsus IV (see Fig. 6e, g) or the tarsal claw contacted the line (e.g., whether she grasped the line).

Attachments to other lines and to the substrate: The process of attaching to an object like a twig or a leaf lasted on average nearly five times longer than attaching to other silk lines (the respective means ± standard deviations were 1.53 ± 0.63 s, $n = 14$, and 0.33 ± 0.26 s, $n = 49$). They also differed in other respects. The spider rocked her abdomen from side to side during 10 of 15 attachments to the substrate (Fig. 6i), but in only 1 of 44 attachments to silk lines. In 10 of 18 attachments to the substrate, the spider immediately turned back toward the direction from which she had come, while no turn backs of this sort occurred in 43 attachments to silk lines.

The spider nearly always bent the tip of her abdomen at least somewhat ventrally when making an attachment (e.g.,
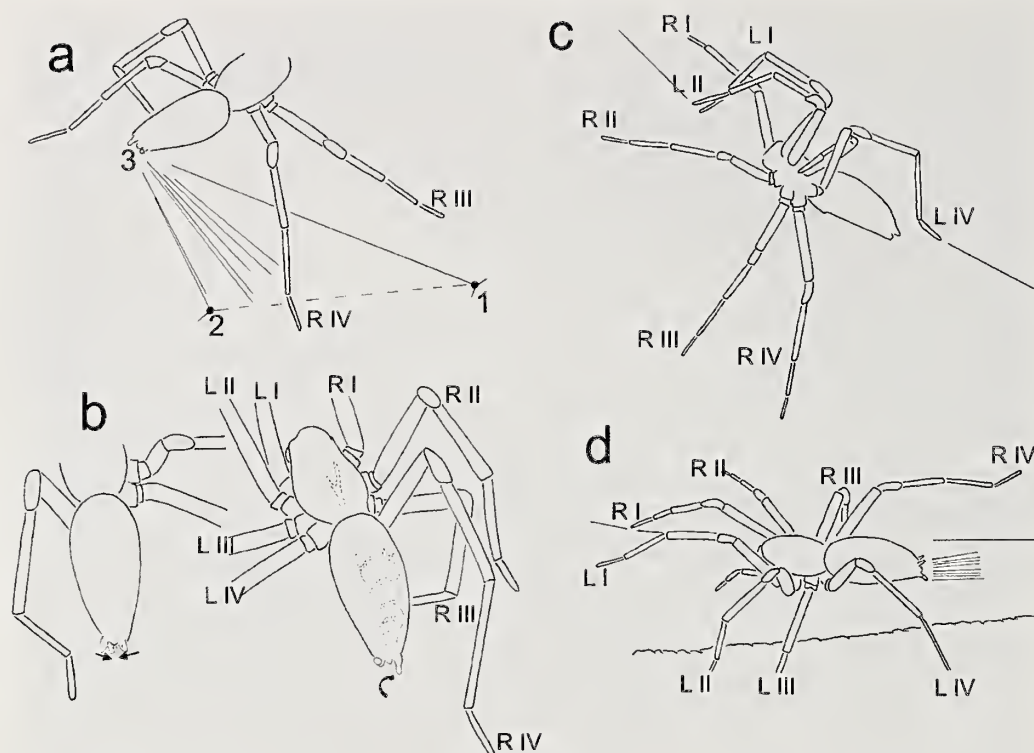
Figure 6, a–d.—Schematic drawings from video images of spiders and portions of lines that glinted in the same frame (or the one immediately preceding or following it) during the early stages of sheet construction (note: drawings include only a fraction of the lengths of the lines, and probably only a subset of the lines that were present). Presumably all "attachments" (dots) were made with piriform silk, but this was not verified. The drawings represent particular cases illustrating patterns which were repeated, but they do not stem from quantitative analyses.(a) The glints on lines seen emerging from the spider's spinnerets illustrate how selective attachments of different lines at different sites can widen the swath of lines that is laid. This spider made attachments to other silk lines at points 1, 2 and 3, in that order. When she made the attachment at 3, some lines stretched directly to the immediately preceding attachment point (2), others stretched directly to the preceding attachment point (1), and still others were apparently attached to the line (or lines) between attachment sites 1 and 2 (dotted line). The spider clapped her posterior lateral spinnerets together four times between making the attachments at points 1 and 2; perhaps the intermediate attachments were produced during these clapping movements. As was typical with other attachments to silk lines, in each of the three attachments the leading posterior lateral spinneret (on the left side at point 3) was extended posteriorly and applied directly to the surface, while the trailing posterior lateral spinneret was directed dorsally and held out of contact. (b) At the moment she made an attachment to other silk lines (drawing on the right), the spider bent her leading leg III (RIII) ventrally to hold the line to which the attachment was being made, and raised the trailing (left) posterior lateral spinneret; she bent her abdomen ventrally and laterally, and rotated it on its longitudinal axis toward this leg (curved arrow at rear of abdomen; note also the position of the markings on the dorsum of her abdomen). Some of these details were executed consistently in other attachments to silk (raise trailing spinneret, lower abdomen), while others were sometimes omitted (twist abdomen, grasp with leg III). Just 3 s before making this attachment, the spider had clapped her posterior lateral spinnerets together (arrows and dotted lines in the drawing at the left). (c) This spider used only her left legs to grasp and walk along a long line while returning to her retreat; legs RII, RIII, and RIV were held extended and nearly immobile below her inclined body, and leg RI waved dorsally toward the line, but only occasionally touched it. The line along which she walked was intact, but was only visible beyond her legs II and IV. (d) In returning toward the retreat after laying a long line to a distant edge of the web early in construction, the spider used her left legs to walk along the upper surface of the branch on which she had walked outward, while her right legs walked along the line(s) she had just laid. Her posterior lateral spinnerets were directed more or less dorsally, while her anterior lateral spinnerets were directed more nearly ventrally, as she produced a swath of approximately parallel new lines.

Fig. 6a, e, g, 7c). In some cases, the spider moved to the far side of an object to which she was attaching an early line; this behavior also occurs in orb-weaving spiders (Eberhard 1990), and probably results in stronger attachments. In some lateral views of a spider making an attachment to the substrate, it was clear that the AL spinnerets pressed against the substrate and moved actively as they did so, presumably depositing piriform lines. In at least some cases the PL spinnerets also pressed against the substrate (Fig. 6g), but there were exceptions (Fig. 6k). We could not observe piriform attachments directly, but because the piriform spigots are located on the AL spinnerets (Santos & Breskovit 2001), it is unlikely for topological

reasons that the aciniform lines from the PL spinnerets were fastened to the substrate with piriform silk during these attachments. Presumably piriform lines attached major or minor ampullate gland lines that were being produced, while the aciniform lines adhered to the substrate (and to other lines – see below) due to their own stickiness soon after emerging from the spigots. In a few cases, the ventral surface of at least one PL spinneret (where the aciniform spigots are located) appeared to be pressed against the substrate (Fig. 6a, b, g).

One clear (and unusual) ventral view of the spider's abdomen while she was making an early attachment in a skeleton web revealed that she clapped her AL spinnerets
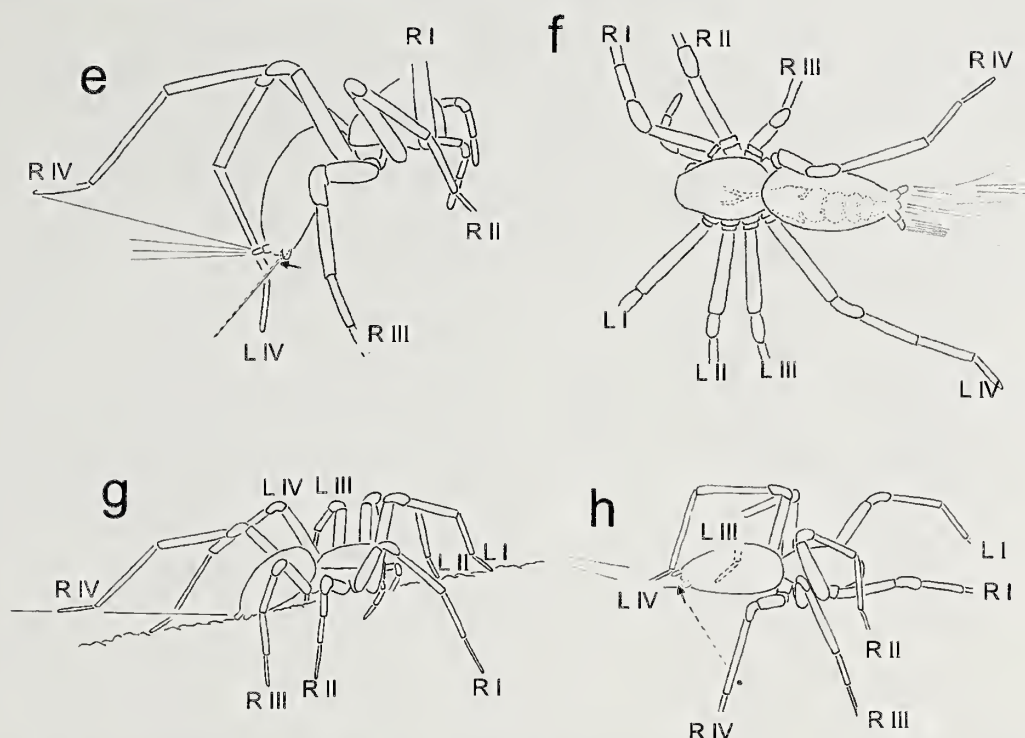
Figure 6, e–h.—(e) Just before attaching to a line, this spider moved her anterior lateral spinnerets ventrally, to apparently pinch this line and then lift it dorsally (dotted lines). One leg (RIV) rested on one or a few of the newly spun lines, but other lines radiated in other directions; no leg III grasped the line to which she was attaching. (f) While walking, the spider spread her posterior lateral spinnerets wide laterally, and produced a wide swath of approximately parallel lines. (g) In making an attachment to a branch, the spider lowered the tip of her abdomen and both her anterior lateral and her posterior lateral spinnerets to contact it. At the moment the attachment was made, the tarsus (or tarsal claw?) of leg RIV rested on the line(s) she was attaching. (h) At the moment she made an attachment to other silk lines, this spider bent her leading leg III (LIII) ventrally to hold a line (apparently the one to which she was making the attachment); the position of the tarsus of this leg is uncertain, as it was not visible. Her left leg IV may have rested on this same line. At the same time, her trailing anterior lateral spinneret moved dorsally (small arrow), probably grasping this line against the other anterior lateral spinneret and bringing it dorsally against her other spinnerets. The attachment immediately preceding this one was at the point indicated by the black dot.

together two or three times just before making an attachment to other silk lines (Fig. 6l). Lateral and dorsal views of spiders revealed similar lateral "clapping" movements of PL spinnerets (Fig. 6b), and dorso-ventral opening and closing movements of the AL and PL spinnerets (Fig. 6e, j) just before and just after attachments to other silk lines, as well as at other times while the spider was building the sheet. These movements might cause aciniform lines to adhere to or to be separated from each other near attachment sites, but we were not able to perceive any pattern of when they occurred. In two cases, it was clear that when the spider flexed her AL spinnerets ventrally, they seized the line to which the spider was going to attach between them, and that they then raised it dorsally toward the other spinnerets; this behavior to our knowledge has never been seen in any other species (Fig. 6e).

When attaching to other silk lines, the spider often (19 of 29 attachments) tilted and rotated her abdomen perceptibly on its long axis toward the leading side (Figs. 6b, 7c). This movement raised the base of the dorsally directed, trailing PL spinneret away from the attachment point, and may have pressed the spigots on the leading side PL spinneret against the lines to which she was attaching.

When making an attachment to other lines, the spider often bent her leading leg III to contact the line to which the attachment was being made anterior to her spinnerets (25 of

43 cases); in four of these cases, the ipsilateral leg IV appeared to also contact this line posterior to her spinnerets. The contact with tarsus III was generally about 3-4 PL spinneret lengths anterior to the spinnerets. The line to which an attachment was being made never glinted, however, so we could not determine whether the ventral surface of the tarsus III or its claw contacted the line. The spider lowered her abdomen toward the line and apparently lifted it toward her spinnerets simultaneously with her leg III (Fig. 6a, b). In contrast, when the spider attached to the silk sheet, leg III made no move to grasp any line near the spinnerets (Fig. 7c).

Usually, when we were able to determine the positions of the lines that emerged from the spinnerets at the moment an attachment was made to other web lines, it was clear that not all of the lines were attached: the positions of lines following attachments also occasionally confirmed that only some but not other lines that the spider was producing were attached at a given point (Fig. 6a), with different lines in a swath of lines that emerged from the spider's spinnerets just before the next attachment coming from radically different directions (Fig. 6a). Confirming this deduction, the trailing PL spinneret was always (*n* = 19 attachments) directed nearly dorsally, while the leading PL spinneret was oriented nearly directly posteriorly (Fig. 6a, b). The spider sometimes raised the trailing spinneret just as the abdomen was being lowered to make the
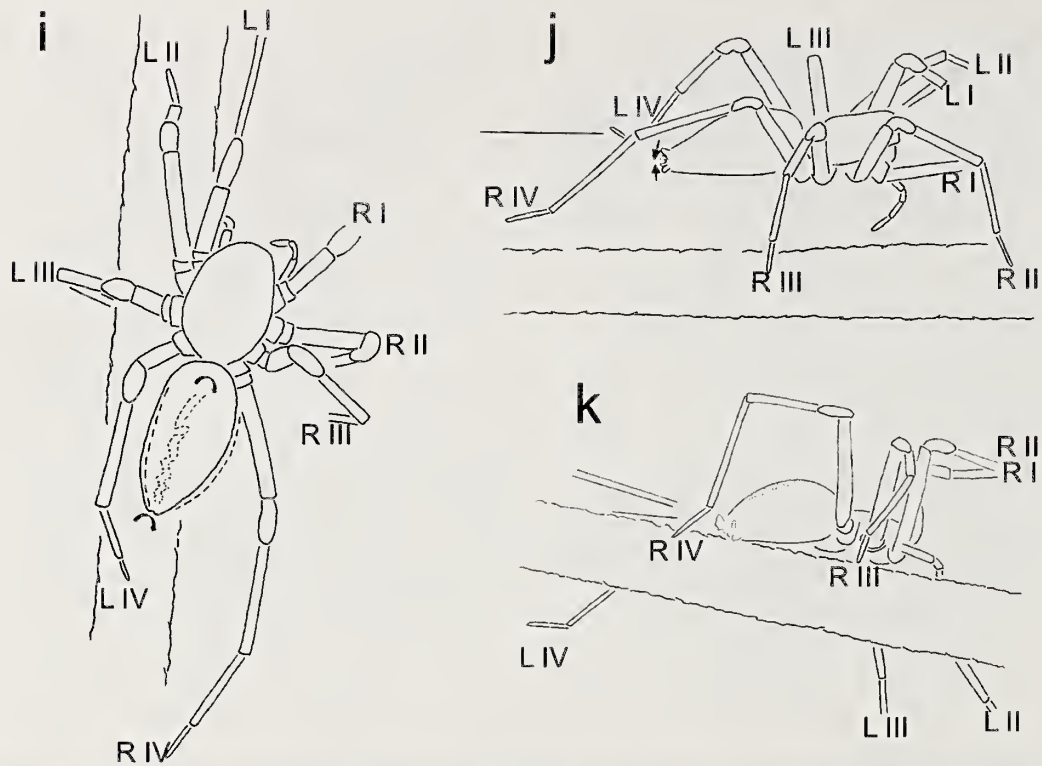
Figure 6, i–k.—(i) The amplitude of the side-to-side rocking movements of the abdomen is illustrated in this spider attaching to a branch (dotted lines were 0.12 s after the solid lines; curved arrows indicate direction of movements). (j) This spider appeared to clap her posterior lateral spinnerets against her anterior lateral spinnerets (small arrows; dotted lines indicate positions 0.09 s later). (k) The spider contacted the branch with her anterior lateral but not with either of her posterior lateral spinnerets as she made an attachment. Her abdomen was tilted on its longitudinal axis, away from the viewer.

attachment. This raised position kept the aciniform lines being produced by the trailing spinneret from the vicinity of the surface (or lines) to which other lines were being attached.

This deduction that aciniform lines from the trailing PL spinneret were not attached was also confirmed directly in a few cases when favorable lighting and viewing angles showed that the multiple lines from the raised PL spinnerets were not attached to the substrate when the AL spinneret contacted the substrate (Fig. 6a, e). Another confirmation came from the fact that, in some other cases, lines apparently emerging from the trailing PL spinneret went lax immediately after an attachment was made and the spider turned toward that trailing side.

In contrast to their relative immobility while the spider walked under a line early in construction and on solid surfaces, the palps made rapid "treading" or bicycling movement while the spider walked on a portion of partially complete sheet.

Later in construction: One individual was filmed for a total of about 30 min during bursts of activity while she made repeated attachments while moving on a sheet that was already partially complete (and also occasionally extended the sheet's edges slightly). Consecutive attachments to the sheet thus usually occurred every one to two seconds; they tended to be approximately one body length apart (Fig. 7), but we did not measure the distances precisely. The temporal pattern of the spider's movements was relatively consistent. After the short pause of about 0.1 s to make an attachment, she moved

quickly forward and somewhat laterally, away from the side on which she had just attached (Fig. 7b); after pausing motionless for several tenths of a second (Fig. 7b), she moved forward again and swung her abdomen laterally to make the next attachment (Fig. 7c). The significance of this abrupt stop-and-go pattern of movement is not clear.

The spider made nearly all attachments (185 of 189) to one side rather than directly behind her as she walked. Sometimes she zig-zagged, making consecutive attachments to opposite sides (Fig. 7), but there was no consistent tendency to alternate (52.9% of 174 consecutive attachments were to alternate sides). Two patterns were evident. When the spider's overall path was curved rather than straight, she tended to attach to the side away from which she was moving; thus, if, for instance, her overall path curved gradually to the right, she consistently attached to the left. When she was near the edge of the sheet, she grasped the lines to which she attached with her leading leg III (Fig. 6b). In contrast, when she was in the middle portion of the sheet, she never grasped lines with leg III (Fig. 7). In many cases, her leading leg III was not even in the vicinity of her spinnerets when the attachment was made. This difference implies that spiders sensed their general locations on the sheets.

Other details of web construction: We found two mature males alone on funnel webs (and another on a web with a mature female). When we removed the sheets and tangles of the solitary males and then checked the sites the following day, one had left but the other was alone on a new web. A mature
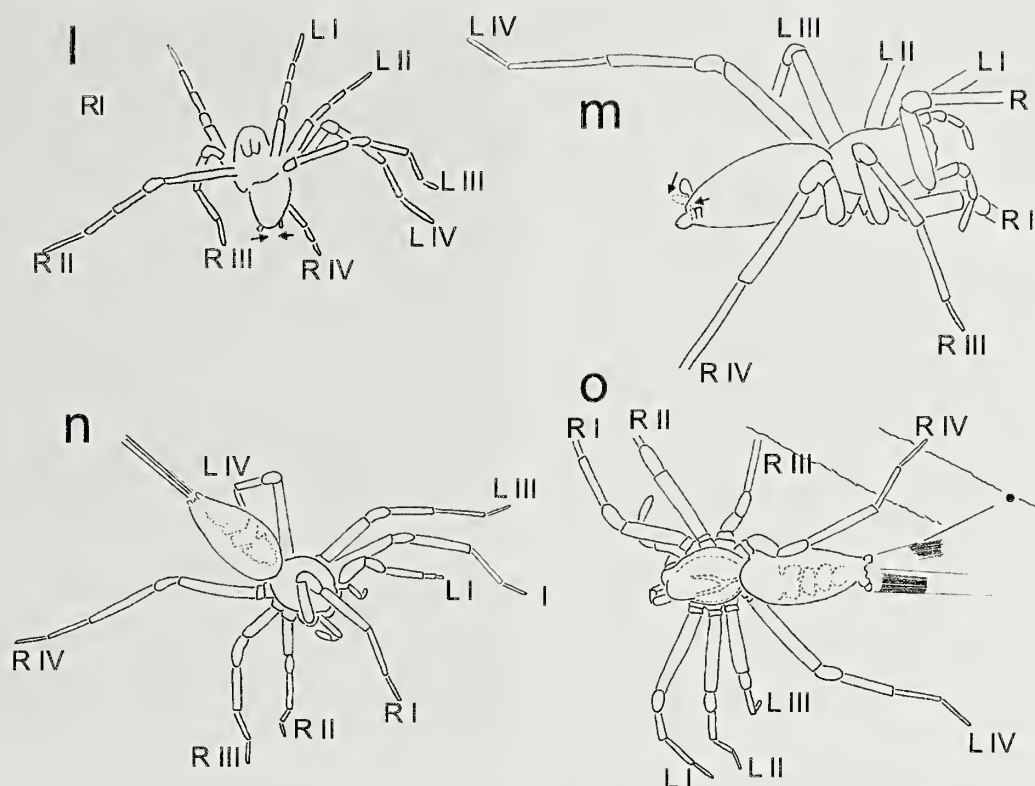
Figure 6, l–o.—(l) A ventral view of the abdomen shows how the anterior lateral spinnerets clapped together (small arrows) just before the spider made an attachment. (m) This drawing illustrates the maximum "gape" that was seen between the anterior lateral and posterior lateral spinnerets; the small arrows and dotted line indicate the positions of the posterior lateral spinnerets just 0.15 s later. (n) This drawing illustrates one limitation of the "glint" technique. A misleadingly low number of lines were visible when this spider was returning to her retreat after having laid a long line. She had not made any attachments on the way back; her posterior lateral spinnerets were directed rearward rather than being spread, and only four lines were visible. Slightly previous to this frame of the recording only two lines were visible; but when she finally made an attachment about 3 s later, her posterior lateral spinnerets were spread apart, and each could be seen to be producing a substantial swath of lines along nearly its entire length. (o) In this case, it was not clear how the swaths of lines from the two, spread posterior lateral spinnerets could be so wide (there were more lines emerging from each than are shown in the drawing). The spider had just attached at the point on the branch marked with a black dot, and at least one line that seemed to originate on her right spinneret was directed toward this point. The swath of fine lines from the left spinneret appeared to run toward the point where the penultimate attachment was made, at least three body lengths farther to the right. But it was unclear where the lines in the swath emerging from her right spinneret were attached.

male also built a sheet and tunnel in captivity. Thus, mature males build webs, at least under certain circumstances.

Sheets appeared to be constructed as units, rather than growing slowly by accretion as do the webs of some spiders, such as the austrochilids *Thaida* Karsch, 1880 and *Austrochilus* Gertsch & Zapfe, 1955 (Lopardo et al. 2004) and the filistatid *Kukulcania hibernalis* (Hentz, 1842) (W. Eberhard pers. obs.). None of six replacement webs that were powdered the morning after they were built (after they had been destroyed the previous day) showed subsequent expansions of the sheet on the following two nights, even though the spiders remained on them. One of these spiders added lines to the middle of the sheet, however, on the second night. The spiders probably added subsequently to the tangles above the sheets. The tangles of ten webs that had been destroyed the preceding afternoon were all skimpy, but some of these grew taller on subsequent days.

Observations in both the field and captivity suggested that the spiders did not excavate retreats, and that their tunnels were built in previously formed cavities. Some of these were well-protected and secure, such as cracks in thick bark and

cavities in tree trunks. Many others in the leaf litter, however, were amongst loose objects and not well-protected, and the spiders could be collected relatively easily.

Prey capture behavior.—Running speed is probably important for *A. castaneus* in prey capture. Three small crickets (body length about one third to one half that of the mature female spiders) that hopped (probably frightened by our approach) onto the sheets and tangles in the field were temporarily retained. At first the insects were immobile; but when they began to move they showed little sign of being entangled, and moved across the sheet with no apparent difficulty. These movements elicited very rapid attacks by the spider, but one cricket nevertheless hopped off the edge of the sheet before the spider arrived. The sheet was relatively fragile, and several holes were left in the sheet at a site where one cricket was captured (Fig. 2d).

These crickets as well as the flies observed in captivity were first bitten, and then, after they were relatively immobile, wrapped using behavior very similar to that of used by *T. radiata* and *Melpomene* sp. to spread a swath of aciniform lines onto the prey (for details, see Barrantes & Eberhard
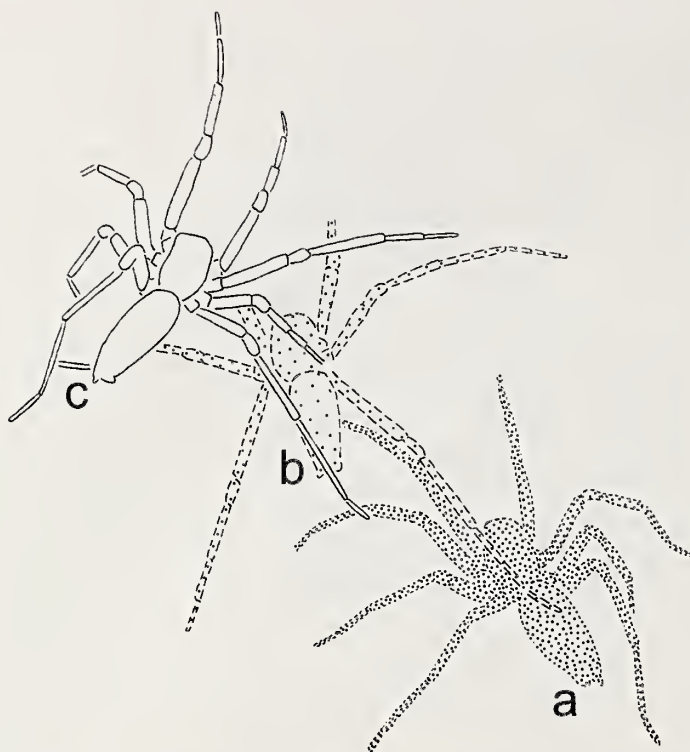
Figure 7.—A schematic view of a typical sequence of movements between two attachments late in sheet construction, when the spider was walking over a sheet and performing presumed sheet filling-in behavior (glints were very uncommon, so the detailed positions of the sheet lines and of the lines that were being produced by the spider were not verified directly; a few legs have also been omitted for clarity). After attaching to the sheet with a sweeping movement of her leading posterior lateral spinneret at position *a* (darker stippling, dotted lines), the spider moved forward and laterally to position *b* (elapsed time 0.43 s), where she remained immobile for 0.43 s (moderate stippling, dashed lines); then she moved farther forward and laterally, and bent her abdomen laterally to make the next attachment at position *c* (elapsed time 0.5 s, no stippling, solid lines). Spiders nearly always paused this way between attachments. The spinneret positions were asymmetrical during attachments, as in earlier stages (e.g., Fig. 6a), but leg III was not bent ventrally to press upward on the lines to which the attachment was made, as occurred in earlier attachments (Fig 6a, b); such a position would have been impossible, because the dense sheet prevented leg III from reaching under it.

2007). The spider began by attaching a swath of wrapping silk to the sheet and then turning slowly in place, laying a swath of fine silk lines from her PL spinnerets. She kept her spinnerets close to the sheet as she turned, and periodically attached the swath of lines to the sheet, thus causing lines to press against the prey. Just as when making attachments to other silk lines during construction, the spider often spread the swath by raising her trailing PL spinneret while she attached wrapping lines to the sheet, and thus avoided attaching the lines from the raised spinneret to the sheet. The spider's legs never touched wrapping lines. The mean duration of each wrapping sequence for the flies was 30 ± 10s, and the mean total duration was 59 ± 5s.

Lines in the sheet under the compound microscope.—The apparent diameters of lines and their orientations varied widely (Fig. 8). The thinnest lines were barely visible at 400x.
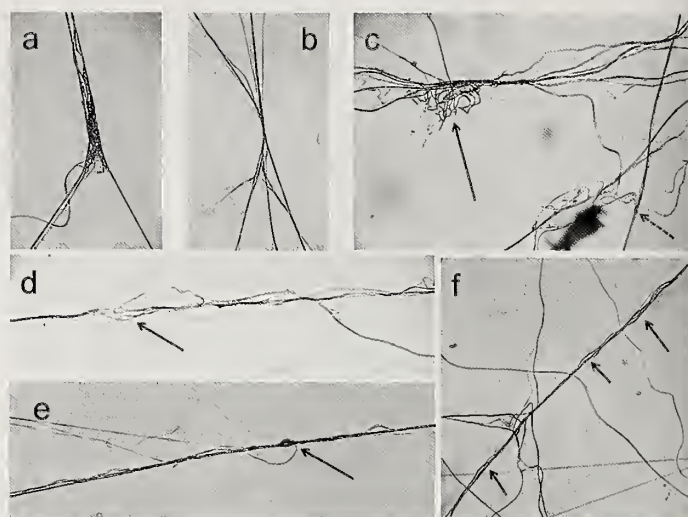

Figure 8.—Different configurations of lines from a newly-built sheet of an *Aglaoctenus castaneus* seen under a compound microscope. (a) A "large" attachment between relatively thick lines. (b) A small attachment that links multiple fine and medium fine lines for a short distance. (e) A large attachment (solid arrow) that brought multiple lines together at the attachment point and also had putative piriform lines splayed apart, apparently on the sheet, and also a small attachment of fine lines (dashed arrow). (d) a thick line that unraveled to reveal that it was composed of many thinner ones. (e) a small droplet of liquid (arrow). (f) a thick line that unraveled in places (arrows) to reveal its multi-strand composition.

We were not able to measure line diameters with confidence because "thick" lines could not be distinguished from compound cables of thinner lines (Fig. 8c). Finer resolution (e.g., SEM photos) will be needed to provide confident measurements of diameters.

As expected from behavioral observations (and despite the "brushed sheet" categorization of *A. castaneus* webs), there were numerous apparent piriform attachments in the sheet. They varied greatly in size. The largest attachments (Fig. 8a) joined relatively thick lines (or eables of lines?), and resembled those of other spiders such as the araneid *Cyrtophora citricola* (Forsskål, 1775) (e.g., Kullmann et al. 1975; Peters 1993). In contrast, the smallest (about $0.1/mm^2$) were barely perceptible thickenings along short segments of thinner lines (Fig. 8b, e). Still others were intermediate in size (Fig. 8c). In one sample sector of 340 mm^2, there were 9 large, 11 medium, and 14 small attachments. In rare cases, there were small droplets associated with piriform attachments (Fig. 8d); these also varied in size. No other droplets were seen at any other sites, suggesting the tentative conclusion that the droplets were material from the piriform gland.

Tengella radiata **(Zoropsidae) webs.**—We can add a few details to the brief descriptions of the webs of *T. radiata* in Eberhard et al. (1993) and Griswold et al. (2005) that permit comparisons with *A. castaneus*. During the day, the spiders were almost always in their tunnels, and not visible. The sheet included both thin and thick lines (or cables of lines) (Fig. 9). The relative number of thick lines was higher than in *A. castaneus* (note the density of visible lines in the unpowdered web in Fig. 9b, where none of the thinner, uncoated lines are visible). Perhaps associated with this great density of thick
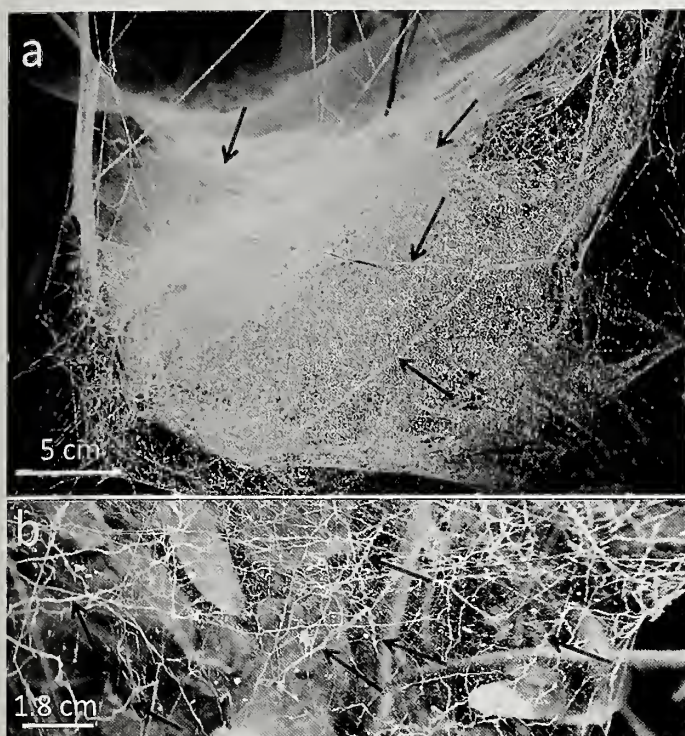
Figure 9.—(a) A portion at the edge of the sheet of a *Tengella radiata* coated with powder, seen from above and the side. The upward curve at the less densely meshed edge is visible at the left and below. Multiple tiers are absent (compare with Fig. 2a of *Aglaoctenus castaneus*, Fig. 11d of *Melpomene sp.*). The arrows indicate long, lax lines covered with cribellum silk that hung free just above the sheet. (b) A close-up view of an un-powdered sheet; arrows indicate lines of cribellum silk that were incorporated in the sheet rather than hanging above it.

lines, there were no perceptible "tiers" of fine lines like those in *A. castaneus* webs (Fig. 9). The sheet was also perceptibly tougher, and prey struggles and capture (Barrantes & Eberhard 2007) often did not result in damage to the sheet.

As in *A. castaneus*, some of the objects to which the sheets and the tangle lines above the sheets were attached would not have been accessible for a spider walking from her retreat; some anchor lines for sheets were >30 cm long. There were highly adhesive cribellum lines in the sheets and also sometimes in the tangle just above the sheet (arrows in Fig. 9a, b). The retreats were consistently associated with cavities such as holes in tree trunks, small, deep cracks in or between stones, holes in the soil, etc. that were more strongly protected than many of those which were used by *A. castaneus*. Tangle construction may resemble that of *A. castaneus* in largely occurring on days subsequent to the day of the original sheet construction (Eberhard et al. 1993). Of 73 webs of older nymphs and mature females, 24.7% had an object (usually twigs, dangling roots, or stems) that protruded through the sheet (as in Fig. 5a, b) (these webs were mostly on the steep sides of a ravine, where the frequency of such objects may have been greater than in webs built in the less cluttered spaces near the trunks of large trees). In some cases, there was a tattered, collapsed older sheet under a newer sheet, as in *A. castaneus*.

Under the compound microscope, lines in the sheets of mature females showed, if anything, a greater variety of diameters (Fig. 10) than in the sheets of *A. castaneus*. Thicker lines sometimes revealed themselves to be cables when they splayed apart into numerous thinner component lines (Fig. 10c). As with *A. castaneus*, few lines were parallel with each other.

In contrast with *A. castaneus*, all piriform attachments were associated with at least one thick or moderately thick line. As in *A. castaneus*, the piriform lines were wrapped around another line in some attachments (Fig. 10f), while in other "open" attachments in which they splayed apart rather than being wrapped around particular lines (or cables of lines) (contrast a, d, e with f in Fig. 10; Kullmann et al. 1975). Perhaps because of the greater size of *T. radiata*, it was possible to distinguish a dense array of very fine lines in some parts of the sheet that were barely perceptible at 400X. The orientations of these very fines lines varied widely; they did not tend to be parallel to each other. The presence of these fine lines (and perhaps even finer lines that could not be resolved) was suggested in many of the open attachment discs where putative piriform lines splayed apart. These had relatively thick lines which were apparently attached to the surface of the sheet (Fig. 10a, d, e). Close examination showed that in many places the putative piriform lines were apparently attached for a short distance to very fine lines in the sheet that were barely resolvable at 400x magnification (arrows in Fig. 10a, d). In these places, several piriform lines ran more or less parallel to each other, with each showing a complementary set of small zig-zags (Fig. 10a, d). In some places, it was clear that these zig-zags occurred where the piriform line intersected one of the very fine lines (arrow in Fig. 10a); in others, there was no visible fine line (but perhaps an even finer, unresolved line?). The parallel orientations of the putative piriform lines were presumably due to the AL spinneret being dragged across the sheet, producing several piriform lines simultaneously. Other piriform attachments had different forms, which included large masses of fine piriform lines joining thick lines (Fig. 10f), and small masses joining smaller lines (Fig. 10b).

A sample sector of 1.96 mm^2 contained 2 large attachments, 11 medium attachments (all but one were open attachment discs), 4 small, and 4 uncertain, giving a conservative estimate of 8.7/mm^2.

***Melpomene* sp. (Agelenidae) webs.**—The webs of *Melpomene sp.* were more similar to those of *A. castaneus* than to those of *T. radiata* in several respects. Most of the lines in the sheet were very fine; there were small tiers of fine, more or less parallel lines just above the sheet (Fig. 11d): the tubular retreats were often at the bases of plants or in the leaf litter (Fig. 11a), and were less consistently located in cavities with rigid walls; the objects to which the tangle lines above the sheets were attached were sometimes inaccessible by walking; and the tangle lines above the sheet included small accumulations of fine lines (Fig. 11b). Spiders observed building webs in captivity did not walk under lines, walking instead on top of the substrate or the sheet that they were building (Rojas 2011) (these observations were in relatively small containers, however, where there were no long lines under which the spiders could have moved). Spiders in the field were generally hidden in the tunnel during the day. The sheets of *Melpomene sp.* often had one or more objects (generally twigs or pine
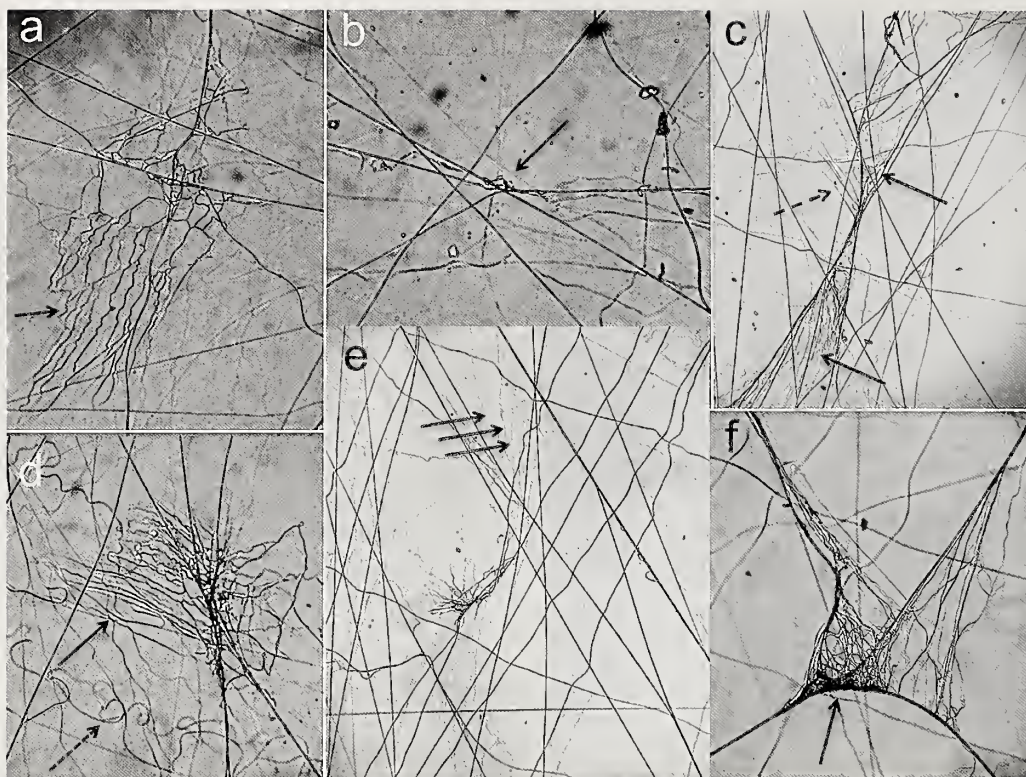
Figure 10.—Different configurations of lines from a newly-built sheet of *Tengella radiata* seen under a compound microscope. (a) A medium "open" attachment with many more or less parallel, wavy putative piriform lines. We believe these are attachments of piriform lines to a sheet. In some places (arrow) the waves in these lines are in register with each other, suggesting that they are produced by their crossing an underlying fine line in the sheet that was too thin to resolve. (b) A small attachment (arrow). (c) Two medium thick lines unravel and are revealed to consist of a large number of smaller lines (solid arrows). A swath of nearly parallel fine (aciniform?) lines is marked with the dashed arrow. (d) a large attachment "open" on the left (arrow) joins relatively thick lines. The loopy lines (dotted arrow) were part of a mat of cribellum lines that were too fine to be resolved. (e) A medium attachment, with many more-or-less parallel fine lines visible, especially in the upper portion of the photo (arrows). (f) large attachment of one thick line to another; most if not all of the putative piriform lines appear to begin or end on the thick lines.

needles) protruding through them (Fig. 11a) (87.5% of 24 webs).

Lines in one new sheet examined under the compound microscope (Fig. 12) resembled those of the other two species in the following respects: lines had a variety of diameters; sometimes a thicker line splayed apart to reveal a cable-like nature; lines showed little tendency to be parallel to each other; and there were both typical, large piriform attachments and apparent "open" attachment discs. It was not possible to determine whether the zig-zag forms of the putative piriform lines were associated with intersections of fine lines as in *T. radiata* (the much smaller size of *Melpomene sp.* made fine lines in their sheets unresolvable). Perhaps more than in the other species, most lines crossed others with no sign of an attachment (Fig. 12b) (though there were some doubtful attachments of finer lines) (Fig. 12c); again the small size of the spiders may have precluded resolution of all the lines. A sample area of 1.56 mm^2 had 4 large, 7 medium and 9–12 small attachments, or about 12/mm^2. They differed from *A. castaneus* and *T. radiata* in that their lines seemed to vary less in diameter, and (for the scale of the web) to have fewer piriform attachments.

When the spider was filling in the sheet, she walked rapidly back and forth across the web, and also returned repeatedly to the retreat, entered the tunnel and immediately turned and

emerged to continue. Only occasionally did the spider appear to make piriform attachments. These occurred in or near the mouth of the tunnel, or near the edge where the sheet was attached to the wall of the container. These attachments were relatively long (the mean for 12 attachments was 0.41 s), and the spider always stopped walking; often in the tunnel, she also thrust her abdomen rearward while making an attachment.

The attachments to the sheet, in contrast, were very brief. As the spider walked across the sheet, she periodically swung her abdomen somewhat laterally, and dabbed it at least slightly toward the sheet. The leading PL spinneret swept rapidly ventrally and/or laterally, apparently brushed against the sheet briefly (the precise position of the sheet was not visible in the recordings, which were taken mostly in dorsal view). In some cases, the spinneret was flexed to point nearly directly downward. The apparent duration of 17 contacts with the sheet averaged only 0.13 s; and this is probably an overestimate, as those extensions of the spinneret that lasted only 1 frame (0.03s) were not included. Frequently the spider continued walking while making a brushing movement with her PL spinneret. The trailing PL spinneret was consistently raised (or in some cases, held more or less horizontally and not lowered), and appeared not to touch the sheet. The low frequency of apparent piriform attachment behavior on the
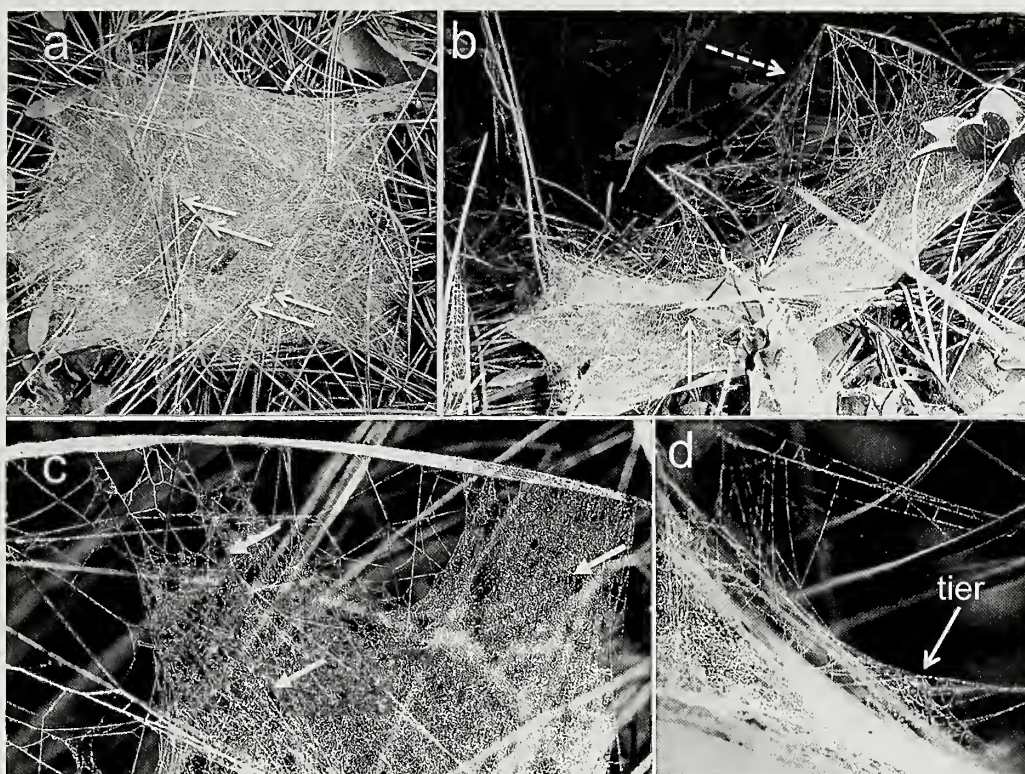
Figure 11.—A horizontal sheet web of the agelenid *Melpomene* sp. at a site where a mat of pine needles covered the ground. (a) View from above, and (b) view from the side. Solid arrows mark pine needles that project through the sheet; the dotted arrow in (b) marks a few of the many lax lines in the tangle above the sheet. (c) Close-up view at one edge of web, taken perpendicular to the sheet. Both probable skeleton lines (longer, straighter and thicker, indicated with arrows) and thinner fill-in lines are visible. (d) A small "tier" is visible just above the sheet in the lateral view.

sheet is in accord with the low frequency of piriform masses seen under the microscope.

DISCUSSION

Comparisons of *A. castaneus* construction behavior with that of other species.—*Behavior associated with laying swaths rather than simple drag lines:* It is likely that *A. castaneus* simultaneously laid both drag lines (presumably of ampullate gland silk from the AL spinnerets) and multiple fine lines (of aciniform gland silk from the PL spinnerets) at many stages of web construction. We could see that swaths of lines emerged from the PL spinnerets (e.g., Fig. 6). We were not able to observe directly that thicker lines which we suppose emerged from the AL spinnerets, as occurred in *A. lagotis* (González et al. 2015), but deduce, from the fact that the frequent attachment behavior observed could only result in piriform attachments of lines from the AL but not from the PL spinnerets, that there were indeed ampullate lines also being laid. Previous descriptions of web construction by other funnel web species, including the lycosids *Hippasa olivacea* (Hingston 1920) and *A. lagotis* (González et al. 2015), and the agelenid *Melpomene sp.* (Rojas 2011), mentioned only a single type of line being produced at a given stage of construction. Producing only a single type of line at a time is well established as the general rule for many other araneomorph web-spinning groups including, as far as we know, all orbicularians (araneoids and deinopoids) (e.g., Eberhard

1982; Vollrath 1992; Zschokke & Vollrath 1995a, b). Because the multiple lines of *A. castaneus* were only visible under favorable lighting conditions, it is possible that the lack of similar observations of swaths of lines in other lycosids is due to incomplete observations, rather than to a lack of simultaneous production of both thick and thin lines. Swaths of lines were also produced during web construction by the mygalomorph *L. macrothelifera* (Eberhard & Hazzi 2012).

Producing a swath of aciniform lines rather than a simple drag line during web construction may explain several other details. The production of swaths of lines by *A. castaneus* was associated with special asymmetrical PL spinneret movements which were used to manage these lines; this spinneret behavior is shared with both the mygalomorph *L. macrothelifera* (Eberhard & Hazzi 2011) and *Melpomene sp.* (A. Rojas, pers comm., this study), but has not been reported previously in the construction behavior of any araneomorph. Spiders routinely elevated the trailing PL spinneret at the moment they moved the abdomen laterally to make an attachment, and thus held the aciniform lines that this spinneret was producing away from the attachment. This behavior resulted in widening the swath (e.g., Fig 6a), a phenomenon which is absent in web construction behavior in many other araneomorphs. Swath widening is presumably advantageous in filling in a sheet web more rapidly and completely with aciniform lines. These movements explain how the spiders produced the paradoxically wide swaths of fine lines seen in photographs of finished *A. castaneus* webs (Fig. 3b, d), which reached widths of up to
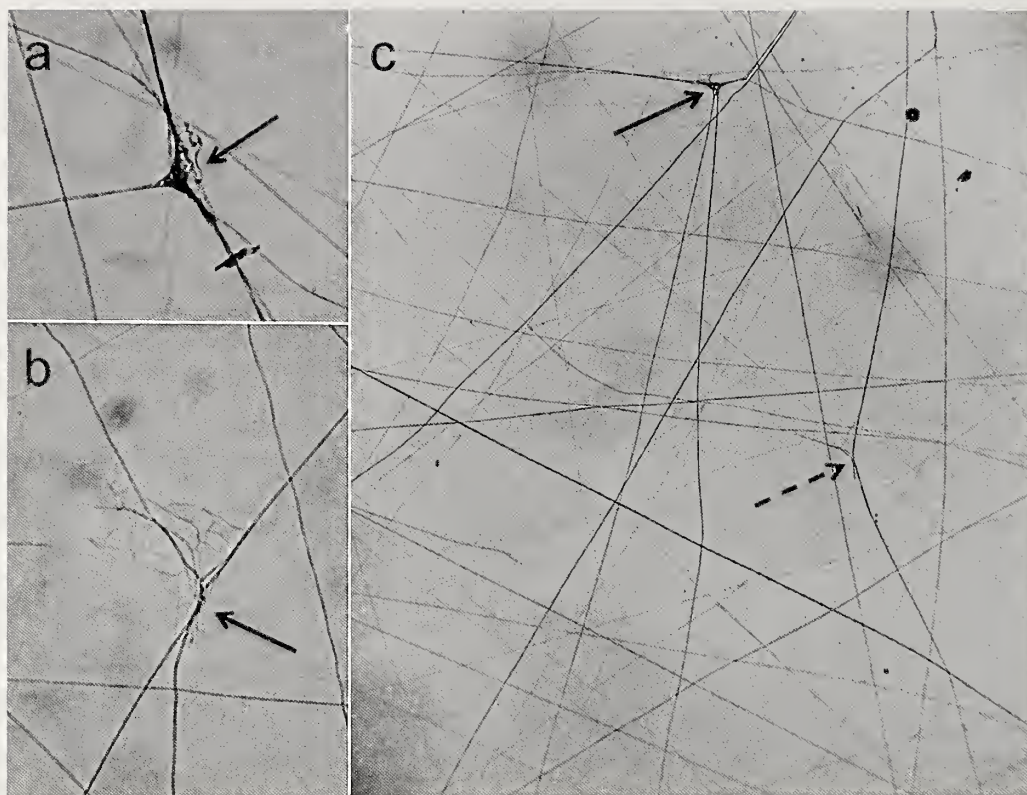
Figure 12.—Different configurations of lines from a newly-built sheet of *Melpomene* sp. seen under a compound microscope. (a) A large attachment (arrow) between two thick lines. (b) A small, possible "open" attachment between smaller lines, in which some of the putative piriform lines are spread apart. (c) A typical overview, showing how most of the lines lacked attachments where they crossed; there is one large attachment between two thick lines (solid arrow) and a small attachment (dotted arrow).

approximately ten times the maximum span of the spider's PL spinnerets. Swath widening during web construction behavior may have originated from prey wrapping (below).

In addition, *A. castaneus* never performed break and reel behavior, as is common in orbicularians (e.g., Marples & Marples 1937; Bradoo 1971; Eberhard 1982; Benjamin & Zschokke 2003; Eberhard & Barrantes 2015). At least in *A. lagotis*, spiders appeared unable to break lines except by physically tugging on them. Break and reel behavior may have been important in the evolution of web designs, because it allows a spider to shift the attachment sites of lines during the construction process, rather than simply accumulating lines to all the sites which she has visited during exploration and construction behavior. In addition, *A. castaneus* never slid tarsus IV along the drag line as do orbicularians (Eberhard 1982; Vollrath 1992; Zschokke & Vollrath 1995a, b), a behavior which may help the spider control the tension on her drag line. Both break and reel behavior and sliding leg IV on the drag line would seem more difficult to perform for a spider which is laying a swath of lines rather than a simple drag line.

The use of legs III and probably IV to hold the lines to which *A. castaneus* was about to attach links this species to araneomorphs (Eberhard 1982) rather than mygalomorphs, and contrasts with the behavior of the diplurid *L. macro-thelifera* (Eberhard & Hazzi 2012) which never used its legs to manipulate lines. *Tengella radiata* resembled *A. castaneus* in

sometimes using one leg III to hold the line to which she was making an attachment against her spinnerets (W. Eberhard, unpubl.). We speculate that using her legs to hold the line to which she is making an attachment improves the preeision with which a spider can apply piriform silk, and was important in the evolution of spider webs because it improved the strength of attachments between silk lines (Wolff et al. 2015). It was not clear, however, whether *A. castaneus* actually grasped lines with her tarsal claws in these situations, as happens in orbicularians, or simply pressed lines with the ventral surface of her tarsus.

Other behavior: An additional behavior which appears to be unique in *A. castaneus* construction behavior, was "one-sided walking", in which only the legs on one side of the spider were used to walk along a single, long, elevated line (Fig. 6c). In our experience, all orbicularian spiders, as well as other web builders such as the pholcids *Modisimus* Simon, 1893 and *Physocyclus* Simon, 1893, use the legs on both sides of the body when walking under a line. The only other spiders we have seen performing one-sided walking were the early nymphs of a mygalomorph (probably of the ctenizid *Ummidia* Thorell, 1875 sp.) that were filmed while walking to a long-distance dispersal site (W. Eberhard, unpub.). The taxonomic distribution of one-sided walking (which we speculate may result from an inability to rotate the tarsus to grasp lines parallel to the leg's longitudinal axis – W. Eberhard, in prep.) is not known.

The order in which lines were produced during sheet construction behavior of *A. castaneus* and *A. lagotis* (González et al. 2015) included early placement of strong lines that formed the skeleton web which was attached to the substrate, and later filling in the sheet with fine lines (Fig. 3). In contrast, the diplurid *Linothele macrothelifera* added sheets of lines from the beginning, without making a preliminary skeleton (Eberhard & Hazzi 2012).

Comparisons of *A. castaneus* webs with those of other species.—*Obstacles in webs:* The webs of *A. castaneus* had objects protruding through the sheet, or bulges in the sheets that were produced by upwardly projecting objects just below the sheet. These barriers probably reduce the speed with which the spider can attack prey, because they would prevent direct dashes to some prey. Similar obstacles occurred in the sheet webs of *T. radiata* and *Melpomene sp.*, and are visible in photographs of the webs of the agelenids *Agelena labyrinthica* (Clerck, 1757) (Nielsen 1931) and *Agelenopsis naevia* (Walckenaer, 1841) (Kaston 1948; Comstock 1948), and the linyphiids *Erigone dentigera* O. Pickard-Cambridge, 1874 and *Microlinyphia pusilla* (Sundevall, 1830) (Kaston 1948). They may be associated with building webs close to the substrate, where there are many potentially interfering objects. Blackledge et al. (2009) mentioned that when spiders evolved webs that were raised farther above or away from the substrate, they would be less "constrained" than those whose webs make more intimate contact with the substrate. They emphasized possible constraints on the forms of webs. We propose that another advantage of elevating webs above the substrate may be reducing the numbers of obstacles in the web that impede rapid attacks on prey.

Webs with such obstacles may represent "errors" in web site selection, when the spider failed to choose a completely open space in which to build her sheet, or was unable to move or remove lines laid early in web construction when she subsequently encountered previously unperceived objects (see discussion of "break and reel" above). In our experience, such errors seem not to occur, or to be rare in aerial sheet weavers such as the theridiid *Parasteatoda tesselata* (Keyserling, 1884), the araneid *Cyrtophora citricola,* the pholcids *Modisimus guatuso* Huber, 1998 and *Physocyclus globosus* (Taczanowski, 1874), and the linyphiid *Dubiaranea* Mello-Leitão, 1943 sp., in which we have seen numerous webs (see also the web photos of e.g., Nielsen 1932; Kaston 1948; Hormiga & Eberhard in prep.); we know, however, of no quantitative studies demonstrating this aspect. Presumably these species differ either because the spiders do a better job of exploring potential web sites for obstacles prior to building their webs, or because their choices of web sites are less constrained by the presence of protected retreat sites as occurs in *A. castaneus* (and to an even greater extent in *T. radiata*) The absence of such obstacles is probably not simply a consequence of these other webs being higher above the substrate, however, but due to the abilities of these spiders to choose more open web sites. There are many possible obstructions such as twigs and leaves high above the ground, and in fact spiders can only build where there are sufficient supports to which to attach their webs. Building a more elevated web is no guarantee that such obstructions will be avoided (as implied by Blackledge et al. 2009). Thus, the existence of fewer obstructions in more aerial webs implies that these other spiders actively avoided obstructions when they chose where to build (though we know of no studies of exploration behavior in any sheet building spider). We speculate that the obstacles in *A. castaneus* webs (and those of *T. radiata* and *Melpomene sp.*) result at least in part from the lack of an ability to shift the positions of lines and their attachment sites, due to their lack of break and reel behavior. This may make it difficult for these spiders to adjust their webs to the presence of obstacles that they only discover after web construction has already begun; it may also explain the occasional apparently superfluous "anchor" lines below the sheet (Fig. 2c).

The multiple small tiers on the sheets of *A. castaneus* were probably produced when spiders laid swaths of fine lines over small concavities in their sheets. Examination of newly constructed sheets in the field showed similar multiple tiers in the agelenid *Melpomene* sp. (Fig. 11) and the diplurid *Linothele macrothelifera* (Eberhard & Hazzi 2012), but not in the zoropsid *Tengella radiata* (Fig. 9). Possibly tiers function by increasing the retention times for prey that have fallen onto the sheet, as presumably occurs with the cribellum lines included in *T. radiata* sheets.

One unexpected observation in *A. castaneus* was that the area covered by the sheet spun on the first night was not extended during the next two nights, even though additional lines were added to the surface of the sheet. The spiders in the field evidently broke and discarded old webs to replace them with new ones. In captivity, *A. lagotis* added lines to thicken but not to extend the sheet after the first night of construction (M. González, pers. comm.); in this case, however, the spiders were confined in small 10×10cm containers, so it is uncertain whether this behavior occurs in the field. Many other non-orb weaving species add to their webs gradually over the space of many days. Captive *Melpomene* sp. added tiers on successive nights and extended the attachments of the edges of their sheets upward on the sides of the container, so the upper surface of the web gradually moved upward (A. Rojas pers. comm.; W. Eberhard unpub.). Again, it is possible that this behavior was an artifact of the size of the containers in which the spiders were kept. *Aulonia albimana* (Walckenaer, 1805) also added silk to the tubular retreat and to the sheet over a period of days, and sometimes built a new sheet on top of an older one (Job 1968).

Construction of prey capture webs by mature males of *Aglaoctenus* (González et al. 2015; present study) also occurs sporadically in other families of web-building spiders, but appears not to be shared with *T. radiata* (Barrantes & Madrigal-Brenes 2008) or *Melpomene sp.* (W. Eberhard, unpub.). Mature males of the lycosid *Aulonia albimana* occasionally built silk tubes as retreats, but apparently did not build sheets (Job 1968), and thus differ from *Aglaoctenus*.

Prey-wrapping and the evolution of web construction behavior.—Prey wrapping in *A. castaneus* corresponds to the "post-immobilization wrapping" behavior of mygalomorphs and araneomorph species (both with and without webs); it probably serves to make the prey package more compact and manageable after the prey is subdued (Eberhard 1967; Robinson et al. 1969; Rovner & Knost 1974; Barrantes & Eberhard 2007; Hazzi 2014). The spinneret movements of *A. castaneus* during wrapping, with the trailing PL spinneret

being raised while the leading one is lowered as the spider was attaching wrapping lines to the substrate or to other lines, and the spider's use of her body rather than leg movements to pull out wrapping silk as she turned, were very similar to prey wrapping in variety of other species, including the theraphosid *Psalmopoeus reduncus* (Karsch, 1880), *Melpomene sp.* (Barrantes & Eberhard 2007), *T. radiata* (Barrantes & Eberhard 2007), and the ctenid *Phoneutria boliviensis* (F. O. Pickard-Cambridge, 1897) (Hazzi 2014). They also resembled movements during sheet construction by the diplurid *Linothele macrothelifera* (Eberhard & Hazzi 2012). This behavior probably serves to widen the swaths of lines applied to the prey (Barrantes & Eberhard 2007; Hazzi 2014). We speculate that swath widening during sheet construction in *A. castaneus* resulted from an evolutionary transfer of ancient prey wrapping behavior to the context of sheet web construction.

"Sheet", "funnel" and "brushed" webs in evolution.—Several recent studies of the evolution of spider web construction behavior that were based on phylogenies generated from morphological and molecular data have used the categories of "sheet" and "brushed sheet" webs in classifying web designs. Our observations reported here have important implications regarding these classifications.

As others have noted (Viera et al. 2007; Blackledge et al. 2009), many previous publications were imprecise in applying the term "sheet web" to characterize a variety of web designs. To pick just one recent example, Murphy et al. (2006) included as "sheets" many diverse structures: the tents that *Dolomedes* Latreille, 1804 spp. build around their egg sacs to protect their nymphs (Comstock 1948; Bristowe 1958); the dense silk retreat embedded in sphagnum moss of *Pirata* Sundevall, 1833 that has an open doorway from which the spider attacks passing prey (Bristowe 1958); the sparse planar arrays of sticky silk in dictynids (some on the substrate, others away from it) (e.g., Nielsen 1931; Griswold et al 2005); and the extensive sheets and silk tubes of *Aulonia albimana* (Job 1968, 1974) and sosippine lycosids. Precision in classifying web forms is obviously crucial in discussing web evolution, especially since nearly all classifications to date have been made on the basis of only the superficial appearance of the web, rather than on observations of construction behavior or the connections between lines in finished webs. Imprecision in terminology can have serious consequences for attempts to trace the phylogeny of web designs. For example, if the claims for homology among the "sheet" webs of Murphy et al. (2006) are over-ambitious, the ease for considering funnel webs as ancestral for Lycosidae is weakened.

A second widely used descriptive term that is also sometimes applied loosely is "funnel web". On the basis of geometry (i.e., a tube connected with a more or less curved plane), this label would apply to webs with a more or less horizontal, planar sheet that is joined with a tubular retreat. This category would include such diverse web designs as the relatively open-meshed sheet webs with adhesive silk of the austrochilid *Thaida peculiaris* Karsch, 1880 (Lopardo et al. 2004) and the psechrids *Psechrus argentatus* (Doleschall, 1857) (Robinson & Lubin 1979) and *Psechrus* Thorell, 1878 sp. (Eberhard 1987b; Zschokke & Vollrath 1995a, b), as well as the dense sheets of fine non-adhesive lines of agelenids, lycosids and diplurids.

A welcome recent consensus appears to be emerging that different types of "sheet" and "funnel" webs need to be distinguished (see Viera et al. 2007; Blackledge et al. 2009). One step in this direction is the proposal of Blackledge et al. (2009) (which was followed subsequently in the phylogenetic studies of Fernández et al. 2014, Bond et al. 2014, and Garrison et al. 2016) to subdivide these categories. Blackledge et al. (2009) distinguished four types of sheets that they appeared to define as follows: brushed —"... brushed silk lines are not specifically and repetitively attached to structural silk threads, but rather lie upon them" and have "... no direct junctions between discreet silk threads ..." and are produced by "... numerous, identical (often aciniform) spigots operating in concert"; irregular ground—"... relatively complex three-dimensional webs that consist of multiple sheets intersecting at various angles and whose overall form tends to follow closely the contours of the substrate to which the webs are attached"; irregular aerial —"... are suspended or free standing ... [and] are relatively amorphous and fill available space in the microhabitat location ..."; and stereotyped aerial—"... are architecturally stereotyped and usually taxonomically distinctive regardless of variation in microhabitat location..." (our uncertainty concerning the definitions is because the text of the "supplementary material" was not exactly parallel to the categories recognized in the figure that documented web evolution). The webs of *A. castaneus*, *T. radiata*, and *Melpomene* sp. are all in the "brushed" category in this classification (J. Coddington pers. comm.).

Unfortunately, placement of different species' webs in these categories has been based only on the overall appearances of webs. There were no observations of behavior; and only one short abstract on a single species (Coddington 2001) was cited concerning connections between threads in webs. The present paper is not the place to attempt a general resolution of how to define "funnel" webs or "brushed sheets". We will, however, take the preliminary step of describing some sets of shared traits, and note some problems in recognizing the "brushed sheet" category.

One species whose webs and behavior seem to fit the criteria for "brushed" is the sheet weaving diplurid *Linothele macrothelifera* (Eberhard & Hazzi 2012), based on evidence from direct observations of construction behavior, close up photos of webs, and the lack of piriform glands in the spiders. The webs of the three araneomorph species of this study, however, clearly do not fit the definition cleanly. The sheets of *A. castaneus* and *T. radiata* had numerous piriform attachments, and during web construction *A. castaneus* paused frequently to press her abdomen to the sheet, apparently making piriform attachments (though, as noted above, these probably probably attached only ampullate and not aciniform lines), rather than brushing her PL spinnerets against the web. Of the three, *Melpomene* sp. was closest to the "brushed" traits. The difficulty in classifying these species arises from the fact that the discrimination was made in quantitative terms (e.g., frequency of piriform attachments in the sheet), but no indication was provided for deciding how infrequent piriform attachments need to be for a web to qualify for inclusion in the "brushed" category.

A second problem is that the term "brushed" conjures up the image of numerous spigots operating in concert, thus

leading to the expectation of many fine lines in the sheet being approximately parallel to each other. Our findings with *A. castaneus* offer only partial confirmation. In some photographs of powdered webs, there were areas with multiple, approximately parallel lines close together (e.g., Fig. 2b, 3b, d). But under the microscope, most lines in the sheets of this species, as well as those in the sheets of *T. radiata* or *Melpomene* sp., were *not* parallel to each other. The overall impression was of disorder (Figs. 8, 10, 12). Presumably the large numbers of non-parallel lines resulted largely from swath widening behavior.

As a first step toward a more natural and informative classification scheme, we point out that the webs of lycosids, agelenids, and zoropsids that are known to date share several traits: a more or less horizontal and planar sheet that is continuous with the walls of a silk tunnel within which the spider shelters; a "skeleton" of thicker lines which is built early, before the sheet is completely filled in with large numbers of very fine lines that are laid onto the skeleton (though in *A. castaneus* the two types of lines are laid simultaneously early in web construction; later behavior is dedicated, at least as far as our fragmentary observations go, more to filling in); relatively frequent piriform attachments between skeleton lines, scarcer piriform attachments involving the fine lines; some relatively parallel orientations of multiple fine lines locally in photographs of powdered webs (though not easily discerned when the sheet is examined under a microscope); highly variable orientations of lines throughout the web (which are due both to the wandering path taken by the spider while laying these lines, and to her lack of inclusion of aciniform lines from her trailing PL spinneret in attachments); and the spider's use of the upper rather than the lower surface of the sheet to move about on her web. A further shared trait is a sparse tangle of thicker lines above the sheet, though the tangle is omitted in some *Aglaoctenus* and *T. radiata* when no appropriate attachment sites are available (González et al. 2015; this paper). The zoropsid differs in also adding sticky lines to the sheet. Many of the details just listed have not been documented in published descriptions of the webs of other species, however, and some behavioral traits (such as possible one sided walking) have yet to be checked in most species. Future discoveries may reveal differences in the details of web design and construction behavior within this group that may suggest different groupings.

Our overall conclusion is that the definition of "brushed" webs is of limited utility. Some webs that were included in this category did not have some of the traits that were used to define the category, and the behavioral observations and microscopic examinations of thread connections in the sheets that would be necessary to test it are lacking in other species. This imprecision in classification raises doubts about some conclusions from some recent studies of the evolution of spider web designs (Blackledge et al. 2009; Fernández et al. 2014; Bond et al. 2014, Garrison et al. 2016). Further behavioral studies and detailed studies of webs are needed to improve the classification schemes used in phylogenetic analyses of the evolution of sheet and funnel webs.

Limitations of this study.—This study was brief and seriously incomplete. Even combining it with that of González et al. (2015), many basic aspects of *Aglaoctenus* web construction have not even been addressed, much less studied carefully. There is no information, for instance, on how the tangle above the sheet is built, on the cues that spiders use to guide building behavior, on the patterns (if any) in the movements during skeleton web construction or filling-in behavior, or on the possible importance of the palps (see Hingston 1920 on *Hippasa olivacea*). Surprisingly, there is still not a single thorough study of funnel web construction in any of the many taxonomic groups that build funnel webs, or of relations between variations in webs and ontogeny, microhabitat charateristics, or reproductive status.

ACKNOWLEDGMENTS

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Morphological and functional diversity of minor ampullate glands in spiders from the superfamily Amaurobioidea (Entelegynae: RTA clade)

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Abstract.   Minor ampullate glands produce fibers that are involved in construction of the complex adhesive band for capturing prey, which is produced by particular cribellate spiders. Despite such a specific role, however, the glands persist even in species where production of cribellate capturing bands no longer occurs. In these species, minor ampullate fibers are instead used to reinforce major ampullate fibers in draglines and capturing webs. The fibers are also used in combination with the aciniform fibrils to make silk for bridging lines – airborne lines used by spiders to allow them to move to points on the substrate where these threads adhere. In this study, we compare the morphology of minor and major ampullate glands in related cribellate and ecribellate groups within spider families of the group traditionally termed the Amaurobioidea, which lies at the base of the RTA clade. We found that the minor ampullate glands are bifurcated in the cribellate members of this group, in particular in the representatives of the families Amaurobiidae, Titanoecidae, Desidae, Amphinectidae and Phyxelididae. In ecribellate representatives, the major ampullate glands are never bifurcated. We found irregularly branched minor ampullate glands in some representatives of the family Agelenidae. In other ecribellates, the glands are either unbranched or they are absent. Thus, bifurcation of the minor ampullate gland seems to be important in determining some aspect of cribellate capturing band formation that is as yet undetermined.

Keywords: Cribellate capturing threads, major ampullate, silk glands, spinnerets

Spiders exhibit a great diversity of silk gland types (Kovoor & Peters 1988). The glands produce silks with a range of different physical properties that are used for different functions. Most attention so far has been paid to the major ampullate glands and their silks. These glands are conspicuous in being the largest in size, and they produce long, thick fibers that are the toughest of all the silk fibers. Spiders use them for walking or dropping draglines, for constructing frames of webs, or as gossamer threads for ballooning (Foelix 2011). The major ampullate glands are usually the only large glands that open onto the anterior (anterior lateral) spinnerets of araneomorph spiders. The large glands that open onto the median (posterior median) spinnerets are called the minor ampullate glands because their morphology resembles that of the major ampullate glands but they are usually smaller in size.

In some cribellate spiders, minor ampullate glands produce fibers that form part of the complex adhesive band used for capturing prey. In this capturing band, a mass of adhesive fibrils produced by cribellar glands is supported by products from three other gland types. The product of the paracribellar glands probably fixes the cribellate fibrils to the fibers from the minor ampullate glands. These fibers also act as springs, tending to contract the whole band (Peters 1987). The last component of the cribellate capturing band is the pair of axial fibers produced by the pseudoflagelliform glands, which open onto the posterior lateral spinnerets. The pseudoflagelliform fibers are straight because they are not combed out using the calamistrum on the hind legs. This is in contrast to products from the previously mentioned glands that open onto the cribellum and the posterior median spinnerets. The pseudo-

flagelliform glands are phylogenetically homologous to the flagelliform glands that produce the axial fibers of the sticky spiral of Araneoidea orb webs.

In contrast to the cribellar and paracribellar glands, which are always absent in ecribellate spiders, minor ampullate glands have not been lost in the majority of cases, even where species no longer produce the cribellate capturing band. Instead, the products of the minor ampullate gland have diversified to fulfil other functions. In spiders from the group Araneoidea, they reinforce the major ampullate fibers in draglines or in construction elements of the webs (Work 1981; Peters & Kovoor 1991). In the family Theridiidae, they have been found to be an important part of prey wrapping silk, where they are held together by aciniform fibers (La Mattina et al. 2008). They are also present, together with other silk types, in orb web stabilimenta of *Argiope* Audouin, 1826 (Tillinghast et al. 1981).

Spiders of the family Nephilidae use minor ampullate fibers to construct the first, nonadhesive spiral of the orb web (Work 1981). This first spiral is necessary for constructing the second, sticky spiral, which is laid by walking on the first one. In the Nephilidae, both spirals stay in the web, but in other orb-weaving Araneoidea, the spiders cut the first spiral out whilst completing the second (Work 1981). Spiders of the genus *Cyrtophora* Simon, 1864 (Araneidae) use only minor ampullate fibers for constructing the meshed-sheet-resembling orb web (Peters 1993). Finally, minor ampullate silk combined with aciniform fibrils is used for bridging lines in the Araneoidea (Peters 1990). Bridging lines are the airborne lines

used by the spider for moving to distant places, once these threads have adhered to a substrate (Peters & Kovoor 1991).

The quantities produced and the physical properties of materials produced by silk glands depend on the size and morphology of each gland. The glands are composed of two main parts, the secretory part and the spinning duct. The secretory part is usually composed of two secretory zones that differ in their transparency and/or colour. They produce different products: the distal zone produces the core and the proximal one produces the coat of the fiber (Kovoor 1987). The secretory part is usually tubular (called the tail), and, in those glands that are required to produce a large amount of silk very rapidly, there is also a wide globular ampulla just before the spinning duct, for storing the silk precursor prior to usage (Kovoor 1987). The spinning duct processes the material from a liquid solution to the solid fiber. In ampullate glands, it starts with a heavily sclerotised funnel, makes two switchbacks *en route* to the spinnerets to form a loop, and the three segments of the loop are all enveloped in a single sheath. The spider's silk glands open on the appendages at the posterior end of its abdomen (spinnerets) through hollow setae called spigots.

The glands that produce large amounts of silk dope usually have an elongated secretory part. However, in some spider groups, the secretory part is branched rather than being simple and elongated. Thus far, bifurcated tail sections of minor ampullate glands have been found in *Amaurobius fenestralis* (Stroem, 1768) (Apstein 1889: plate 5, Fig. 75) and *A. ferox* (Walckenaer, 1830) (Atanasiu-Dumitresco 1941: plate 2, Figs. 4, 5). Irregularly branched tails of both the major and minor ampullate glands have also been found in some Agelenidae, such as *Eratigena atrica* (C.L. Koch, 1843) (Kiesow-Starck 1932: Figs. 6–8 under the name *Tegenaria atrium*; Kovoor 1976, 1987) and *Agelena labyrinthica* (Clerck, 1757) (Apstein 1889: Fig. 67; Kiesow-Starck 1932: Figs. 18, 20). The precise gland morphology in the latter remains uncertain, however, because other studies have found simple ampullate glands in this species (Kovoor 1976: Fig. 1). Misidentification of the studied material, or observation of only some of the ampullate glands, might explain this apparent anomaly. Morphological features of the spinning glands are not mentioned in other studies of similar species, such as *Agelena limbata* Thorell, 1897 (Park & Moon 2002). Irregularly branched tails in ampullate glands have also been found in *Tegenaria ferruginea* (Panzer, 1804) although the results were erroneously presented under the name *T. domestica* (Clerck, 1757) in Johansson (1914) and Kiesow-Starck (1932: Fig. 11). Notwithstanding uncertainty regarding species identity, the morphology of the silk glands is certainly variable even within the family Agelenidae. For example, simple ampullate glands have been found in other agelenid species such as *T. domestica* (Johansson 1914: Fig. 5; Kiesow-Starck 1932: Figs. 12–14; both authors refer to this species under the name *T. derhami* Hansen, 1882; Atanasiu-Dumitresco 1941: Figs. 9–10).

The aim of this study is to compare the morphology of the minor ampullate glands in closely related cribellate and eeribellate groups of spiders, where the phylogenetic relationships indicate that the cribellate band has been lost more than once. These species come from within the Amaurobioidea, the monophyletic sister clade to the clade containing Dionycha

Figure 1.—Bifurcated minor ampullate glands of cribellate representatives of the superfamily Amaurobioidea. Either "one side" or "both sides" of the abdomen, split medially, are depicted. (A) *Themacrys silvicola* female, the side branch is approximately the same length as the axial one, additional branching is apparent. (B) *Vidole sothoana* female, the side branches are slightly shorter than the axial one, the side branch of the left gland is unusually turned to the front, additional branching is apparent. (C) *Metaltella simoni* female, the ampulla is completely missing, the proximal secretory zone is much darker than the distal one, the side branches are much smaller than the axial ones, the side branch of the right gland is not seen because it is behind the axial one. (D) *Amaurobius ferox* female, the ampulla and the constriction proximally from it are apparent, the side branch is approximately the same length as the axial one. (E) *Amaurobius ferox* male; in comparison with the conspecific female the storage ampulla is smaller, but besides that other reductions are not apparent. (F) *Amaurobius jugorum* female, constriction proximally from the ampulla is apparent, the side branch is approximately the same length as the axial one, additional branching is apparent. (G) *Callobius bennetti* female, the ampulla is completely missing, the side branches are shorter than the axial ones. (H) *Phryganophorus candidus* female, the ampulla and the constriction proximally from it are apparent, both branches are approximately the same length, they direct to the sides, the duct is relatively long, the loop covers a third of the distance between the funnel and the spinneret (marked by an arrow). (I) *Titanoeca quadriguttata* female, initially both branches direct to the front and then the side one turns backwards, the side one is longer than the axial one, additional branching is apparent. The scale bars represent 200 μm.

Figure 2.—Minor ampullate glands of representatives of Agelenidae spiders. Either "one side" or "both sides" of the abdomen, split medially, are depicted. (A) *Coelotes terrestris* female, two glands of almost the same size open on each posterior median spinneret, the ampulla is completely missing, the distal secretory zone is lighter and much shorter than the proximal one. (B) *Wadotes calcaratus* female, one gland from the couple was lost during dissection, the ampulla is completely absent, the distal secretory zone is shorter and lighter than the proximal one. (C) *Histopona torpida* female, the proximal secretory zone is darker than the distal one. (D) *Tegenaria campestris* male, apparent are the long distal secretory zone and the short proximal zone. (E) *Tegenaria silvestris* female, apparent is the constriction proximally from the ampulla, the proximal secretory zone is darker than the distal one, the secretory part is enlarged by elongation. (F) *Tegenaria ferruginea* juvenile female, the secretory part is enlarged by bifurcation. (G) *Tegenaria domestica* female. (H) *Eratigena agrestis* female, the secretory part is enlarged by irregular branching. (I) *Eratigena atrica* female, the secretory part is enlarged by irregular branching, the dwarf gland is marked by an arrow. (J) *Agelena labyrinthica* female; (K) *Allagelena gracilens*, female; (L) *Agelenopsis pennsylvanica* female. The scale bars represent 200 μm.

and Lycosoidea (see Fig. 5 in Miller et al. 2010, and Fig. 1 in Garrison et al. 2016). We also compare these glands with the major ampullate glands within each species. We identified a bifurcated secretory part of the minor ampullate glands in

cribellate spiders from the families Amaurobiidae, Titanoecidae, Desidae, Amphinectidae and Phyxelididae. Furthermore, we found ramified (more than two branches) secretory parts of both major and minor ampullate glands in some Agelenidae, a
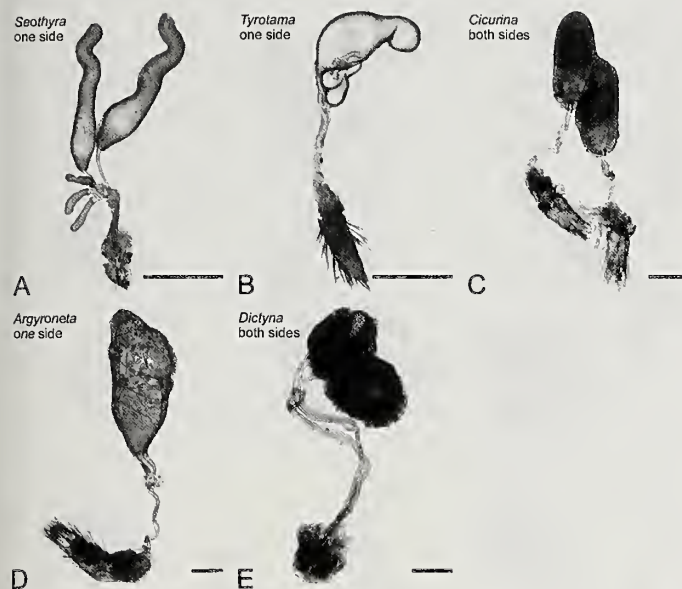
Figure 3.—Simple minor ampullate glands of the representatives of the superfamily Amaurobioidea and its outgroups. Either "one side" or "both sides" of the abdomen, split medially, are depicted. (A) *Seothyra schreineri* juvenile female, two functional and probably four (the fourth is not visible) accessory glands. (B) *Tyrotama australis* female, the ampulla and the two accessory glands are apparent. (C) *Cicurina cicur* female, the secretory part is not elongated (the tail is missing), the distal secretory zone is darker than the proximal one. (D) *Argyroneta aquatica* subadult male. (E) *Dictyna uncinata* female, the secretory part is not elongated (the tail is missing), the duct is relatively long, the loop covers one third of the distance between the funnel and the spinneret. The scale bars represent 200 μm.

spider group that no longer has a cribellum (Miller et al. 2010). In other Agelenidae, Dictynidae, and also the studied outgroups of Amaurobioidea, Hersiliidae and Eresidae, the minor ampullate glands are simple, i.e., without bifurcation. In the hahniid genera *Antistea* Simon, 1897 and *Cybaeus* L. Koch, 1868 and the studied representatives of Zodariidae, another outgroup of Amaurobioidea, the minor ampullate glands are absent. We use our data to discuss the functional significance of bifurcation of the minor ampullate glands in cribellate spiders.

METHODS

The material studied (Table 1) includes representatives of ten spider families that are at the base of the RTA clade, the majority of which are usually included in the superfamily Amaurobioidea. We also studied representatives of the families Eresidae and Hersiliidae as outgroups to the RTA clade.

The spinning glands of fresh spiders were dissected in embryo dishes using physiological solution (0.9% aqueous solution of sodium chloride) and viewed under an Olympus SZX12 stereomicroscope. They were subsequently transferred in a drop of physiological solution onto a microscope glass slide with a small prefabricated circular impression and photographed under a Nikon Eclipse 80i light microscope. Voucher specimens are deposited in the Crop Research Institute, Prague (Czechia).

Figure 4.—Major ampullate glands of the cribellate representatives of the superfamily Amaurobioidea. Either "one side" or "both sides" of the abdomen, split medially, are depicted. (A) *Themacrys silvicola* female. (B) *Vidole sothoana* female. (C) *Metaltella simoni* female. (D) *Amaurobius ferox* female. (E) *Amaurobius ferox* male. (F) *Amaurobius jugorum* female. (G) *Callobius bennetti* female. (H) *Phryganophorus candidus* female, the secretory part is enlarged by irregular branching. (I) *Titanoeca quadriguttata* female. The scale bars represent 200 μm.

RESULTS

Minor ampullate glands.—We found no minor ampullate glands in *Zodarion* Walckenaer, 1847, *Psammorygma* Jocqué, 1991, *Cybaeus*, and the species from the Hahniidae. In the majority of studied species, only one pair of minor ampullate glands was present (Figs. 1, 3C–E). More pairs were observed in outgroup species (Eresidae, Hersiliidae; also in representatives of the clades Lycosoidea and Dionycha, which were not included in this study) and the Agelenidae. In the Agelenidae, there were either two pairs of glands of almost the same size (*Agelenopsis* Giebel, 1869, Fig. 2L; *Coelotes* Blackwall, 1841, Fig. 2A; *Wadotes* Chamberlin, 1925, Fig. 2B–here only one gland is shown; *Eratigena* Bolzern, Burckhardt & Hänggi, 2013, Fig. 2H & I), or one large and two smaller "dwarf pairs" (*Histopona* Thorell, 1869, *Tegenaria* Latreille, 1804, Fig. 2C–G; *Agelena* Walckenaer, 1805, *Allagelena* Zhang, Zhu & Song, 2006, Figs. 2J & K). Dwarf glands can easily be lost during dissection and are thus not always apparent (e.g., Fig. 2). A system consisting of three pairs of co-occurring glands has previously been shown to allow production of fibers, even during proecdysis (Townley et al. 1993).
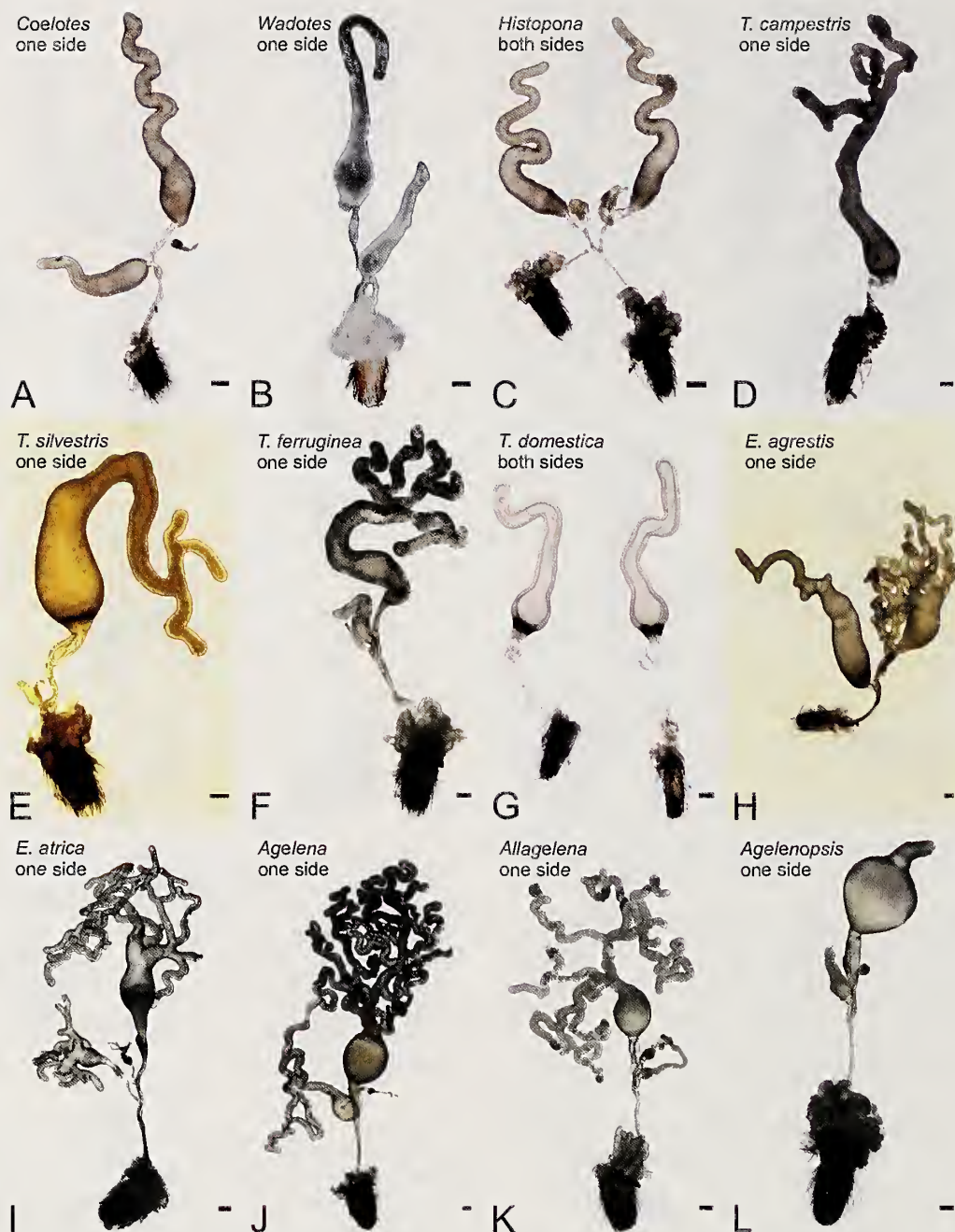
Figure 5.—Major ampullate glands of the representatives of Agelenidae spiders. Either "one side" or "both sides" of the abdomen, split medially, are depicted. (A) *Coelotes terrestris* female. (B) *Wadotes calcaratus* female. (C) *Histopona torpida* female. (D) *Tegenaria campestris* male. (E) *Tegenaria silvestris* female. (F) *Tegenaria ferruginea* juvenile female. (G) *Tegenaria domestica* female. (H) *Eratigena agrestis* female. (I) *Eratigena atrica* female. (J) *Agelena labyrinthica* female. (K) *Allagelena gracilens* female. (L) *Agelenopsis pennsylvanica* female, the distal (unbranched) part of the secretory zone is broken. In D–F and H–K, the secretory part is enlarged by irregular branching. The scale bars represent 200 μm.

The minor ampullate glands were found to possess two secretory zones in the studied species. The zones differed in visual parameters (transparency and colour). The proximal zone is usually smaller than the distal zone (e.g., Fig. 2D), although in representatives of the agelenid subfamily Coelotinae (*Coelotes*, Fig. 2A; *Wadotes*, Fig. 2B), it is significantly larger.

The proximal portion of the secretory part is in some cases widened to form a storage ampulla (e.g., Figs. 1D & H, 2E &

H). The most proximal part of the ampulla is sometimes constricted (e.g., Figs. 1D & F, 2E). In the majority of the studied species, the minor ampullate glands have an unbranched tubular tail (Figs. 2A–E, 3, Table 2), but in some species the tail is bifurcated (Fig. 1). One branch usually continues anteriorly in the axis of the gland (axial branch), whereas the second one grows to the side (side branch) and then it turns backwards, toward the spinneret (Fig. 1A–G & I). The side branch is either shorter (Fig. 1B, C & G) or of the
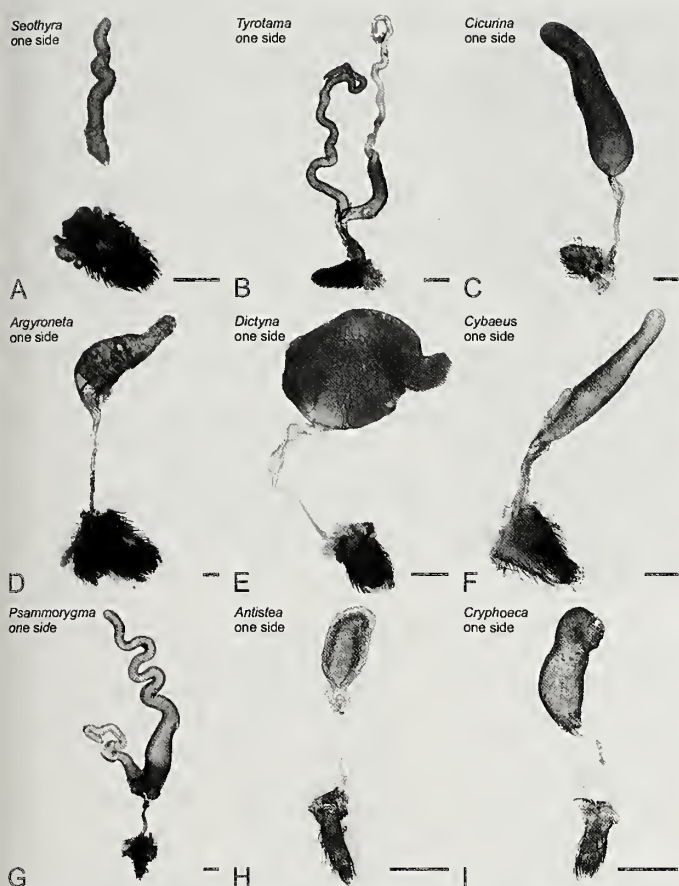
Figure 6.—Major ampullate glands of the representatives of the superfamily Amaurobioidea and its outgroups. Only "one side" of the abdomen, split medially, is depicted. (A) *Seothyra schreineri* juvenile female. (B) *Tyrotama australis* female. (C) *Cicurina cicur* female. (D) *Argyroneta aquatica* subadult male. (E) *Dictyna arundinacea* female. (F) *Cybaeus angustiarum* female. (G) *Psammorygma* sp., female. (H) *Antistea elegans* female. (I) *Cryphoeca silvicola* male. The scale bars represent 200 μm.

same length as the axial branch (Fig. 1D, F & H). In a minority of cases, it is longer (Fig. 1I), and occasionally both branches protrude to the side (Fig. 1H). In *T. ferruginea* the tail has 2–3 branches in juveniles (Fig. 2F) and 3–4 branches in adult females, while in *Eratigena* and the primary glands of *Agelena* (Fig. 2J) and *Allagelena*, it is multiply branched (Fig. 2H, I & K). In some Amaurobiidae, Phyxelididae and Titanoecidae, we found slight marks of additional branching (Fig. 1A, B, F & I).

The spinning duct is usually relatively short and carries a short loop on its most distal part, close to the duct funnel, although in Dictynidae (Fig. 3E), Desidae (Fig. 1H) and some Amaurobiidae (Fig. 1D–G) the loop is longer, covering more than one third of the distance between the funnel and the spinneret.

Major ampullate glands.—In contrast to the minor ampullate glands, the major ampullate glands are present in all the studied species. In the majority of species, they are well developed, although there are exceptions; for instance in *Seothyra* Purcell, 1903 (Eresidae), they are relatively small and are hidden within the piriform glands (Fig. 6A). There are three major ampullate glands attached to one anterior lateral spinneret: the first gland is large and functional, and the second is very small and reduced in size. The third gland is intermediate in size, ranging from almost the same size as the large gland (Figs. 4D & F, 5H, 6B) to approximately the size of the small gland (Figs. 4E & 4I, 5C & F). The major ampullate glands appear to possess two secretory zones. The zones differ in visual parameters such as transparency or colour, the B zone being lighter or darker than the A zone, or brownish. Zone A is always white or transparent. The observed colouration does not seem to be constant and probably depends on the actual stage of epithelial cells in their secretory cycle (the height of the epithelial cells and presence of product droplets inside the cells changes during the cycle). The proximal zone (zone B) is always smaller than the distal zone (zone A), and constitutes less than one quarter of the length of the secretory part (Figs. 4–6).

In the majority of the studied species, the proximal portion of the secretory part is widened to form a storage ampulla (this was most apparent in *Tegenaria*, *Eratigena* and Desidae; Figs. 4H, 5E & G–I). In a small number of cases, the secretory part of the gland is a tube of uniform width (Eresidae, Hersiliidae; Fig. 6A & B). In the majority of the studied species, the major ampullate glands have an unbranched tubular tail. In some species, it is slightly elongated (*Cicurina* Menge, 1871, Fig. 6C), but, in most of the others, the elongation is extensive (most apparent, for example, in *Psammorygma*; Fig. 6G). In order to harbour the entire length of the tail in the limited space available in the abdomen, the tail is often undulated. In some *Tegenaria* species and, in the family Desidae, the tail is sparsely branched (Figs. 4H, 5D–F), whereas in *Eratigena* and the primary glands of *Agelena* (Fig. 5J) and *Allagelena*, the tail is densely branched (Figs. 5H, I & K). The spinning duct of the major ampullate gland is relatively short and carries a short loop on its most distal part, which lies close to the duct funnel.

DISCUSSION

We have shown that bifurcated minor ampullate glands are restricted almost exclusively to cribellate representatives of Amaurobioidea, *sensu* Garrison et al. (2016). The bifurcation might simply be a means through which more material can be produced, but the co-occurrence of cribellar glands with bifurcated minor ampullate glands suggests that bifurcation may be important for correct formation of the cribellate band structure.

A comparison between the morphology and function of the minor and major ampullate glands in cribellate and ecribellate Amaurobioidea spiders provides some insight. In the cribellate spiders that were studied, the products of the major and minor ampullate glands presumably serve different functions. The major ampullate fibers are used for draglines, ballooning or construction of webs. The minor ampullate glands presumably produce undulating fibers supporting cribellate fibrils in the adhesive bands for capturing prey (shown for Deinopidae and Eresidae – Peters 1992a, b).

The morphology of the major and minor ampullate glands in cribellate spiders was also found to differ – the major ampullate glands being simple (Fig. 4), in contrast to the bifurcated nature of their minor ampullate counterparts (Fig. 1). Bifurcation of the secretory part might produce a fiber that

Table 1.—The spider material studied. Family classification follows World Spider Catalog (2016). *Cicurina*, *Argyroneta* and *Cybaeus* after Murphy & Roberts (2015). Higher taxa after Miller et al. (2010).

Family/Species	Higher taxon	Material	Date of dissection	Site
Cribellum present				
Amaurobiidae	RTA: Amaurobioidea			
Amaurobius fenestralis (Stroem, 1768)		3♂, 4♀	19-Nov-15	Czechia: Hostivice
Amaurobius ferox (Walckenaer, 1830)		5♀	4-Dec-14	Czechia: Bítov
Amaurobius jugorum L. Koch, 1868		4♀	3-Jun-14	Czechia: Bítov
Callobius bennetti (Blackwall, 1846)		2♀	6-Jul-14	USA: OH: Licking County, Dawes Arboretum
Amphinectidae	RTA: Amaurobioidea			
Metaltella simoni (Keyserling, 1878)		1 ♂, 1♀	6-Jul-14	USA: CA: Riverside
Desidae	RTA: Amaurobioidea			
Phryganoporus candidus (L. Koch, 1872)		1 ♀	28-Jul-14	Australia: Kinchega National Park
Dictynidae	RTA: Amaurobioidea			
Dictyna arundinacea (Linné, 1758)		2♀	18-May-17	Czechia: Praha-Ruzyně
Dictyna uncinata Thorell, 1856		4♀	19-May-15	Czechia: Praha-Ruzyně
Eresidae	Outgroup of RTA			
Seothyra schreineri Purcell, 1903		2♀, 2 juv.	15-Dec-15	South Africa: Bankfontein
Phyxelididae	RTA: Amaurobioidea			
Themacrys silvicola (Lawrence, 1938)		2♀	3-Nov-15	South Africa: Royal Natal National Park
Vidole sothoana Griswold, 1990		2♀	3-Nov-15	South Africa: Amanzi Private Game Reserve
Titanoecidae	RTA: Amaurobioidea			
Titanoeca quadriguttata (Hahn, 1833)		4♀	6-Jun-14	Czechia: Havraníky
Titanoeca schineri L. Koch, 1872		2♀	6-Jun-14	Czechia: Hodonín
Cribellum absent				
Agelenidae	RTA: Amaurobioidea			
Agelena labyrinthica (Clerck, 1757)		1♀	13-Jul-16	Czechia: Praha-Ruzyně
Agelenopsis pennsylvanica (C.L. Koch, 1843)		2♀	10-Sep-09	USA: OH: Akron
Allagelena gracilens (C. L. Koch, 1841)		1♀, 5 juv.	12-Jul-16	Czechia: Valtice
Coelotes terrestris (Wider, 1834)		4♀, 2 juv.	19-Nov-14	Czechia: Podmolí
Eratigena agrestis (Walckenaer, 1802)		5♀	18-Nov-14	Czechia: Lukov
Eratigena atrica (C. L. Koch, 1843)		5♀	5-Jun-14	Czechia: Hostivice
Histopona torpida (C. L. Koch, 1837)		4♀	18-Nov-14	Czechia: Lukov
Tegenaria campestris C. L. Koch, 1834		4♀	18-Nov-14	Czechia: Lukov
Tegenaria domestica (Clerck, 1757)		3♀	6-Jun-14	Czechia: Znojmo
Tegenaria ferruginea (Panzer, 1804)		5 juv., 2♀	7-Dec-15	Czechia: Praha-Ruzyně
Tegenaria silvestris L. Koch, 1872		4♀	4-Nov-15	Slovenia: Orehek, Žegnana jama
Wadotes calcaratus (Keyserling, 1887)		2♀	13-Jul-14	USA: OH: Licking County, Dawes Arboretum
Dictynidae	RTA: Amaurobioidea			
Argyroneta aquatica (Clerck, 1757)		1 ♂, 5♀	5-Nov-14	Czechia: Doksy
Cicurinidae	RTA: Amaurobioidea			
Cicurina cicur (Fabricius, 1793)		4♀	21-May-15	Czechia: Praha-Ruzyně
Hahniidae	RTA: Amaurobioidea			
Antistea elegans (Blackwall, 1841)		7♀	4-Oct-16	Czechia: Staré Splavy
Cryphoeca silvicola (C. L. Koch, 1834)		1♂	4-Oct-16	Czechia: Pec pod Sněžkou
Cybaeus angustiarum L. Koch, 1868		5♀	19-Sep-15	Czechia: Pec pod Sněžkou
Hersiliidae	Outgroup of RTA			
Tyrotama australis (Simon, 1893)		2♀, 1 juv.	15-Dec-15	South Africa: Bankfontein
Zodariidae	RTA: outgroup of Amaurobioidea			
Psammorygma sp.		2♂, 2♀	13-Jan-16	South Africa: Ndumo Game Reserve
Zodarion germanicum (C. L. Koch, 1837)		1 ♂, 2♀	21-May-15	Czechia: Praha-Liboc

is asymmetric in its cross-section, with one side containing more product from the adjacent branch of the secretory part. The asymmetry of the fiber could enhance its undulating properties and/or could secure different adhesion forces on its different sides. The latter seems unlikely, since presumably the products of both branches are ensheathed by the product of the proximal portion of the secretory epithelium. Alternatively, the undulating of these fibers could also be caused simply

by combing the cribellate band out using the calamistrum. If the bifurcation of the gland is an adaptation for the production of asymmetric fibers, the epithelium in the different branches, as well as their products, should differ. Furthermore, the sides of the resulting fiber should express different physical properties. We did not notice any visual differences (for example, different colouration or transparency) between the epithelium of particular branches (Fig. 1). Both branches contain transparent liquid crystalline material.

Surprisingly, we did not find any obvious reduction of the minor ampullate glands in adult males when compared with females and juveniles (compare scale bars in Fig. 1D & E). Such a reduction might be expected, because adult males do not have functional cribellar glands, and thus cannot produce adhesive capturing bands (which are supported by fibers from the minor ampullate glands in females and juveniles). Our finding of unreduced, thus probably functional, minor ampullate glands in adult amaurobiid males suggests that minor ampullate fibers may fulfil some additional function in adult males.

Cribellar glands are believed to have been lost independently several times in spider evolution (Spagna & Gillespie 2008; Miller et al. 2010; Dimitrov et al. 2016). The species described in this study represent at least four cases of independent loss of the cribellum: two outgroups of Amaurobioidea – Hersiliidae (with the cribellate sister family Oecobiidae) and Zodariidae (with the cribellate sister family Penestomidae), and *Argyroneta* Latreille, 1804 (ingroup of the otherwise cribellate Dictynidae) and the clade composed of Agelenidae, Hahniidae and Cicurinidae (*sensu* Murphy & Roberts 2015, with the cribellate sister family Dictynidae). In addition to the cribellar glands, the paracribellar glands and frequently also the pseudoflagelliform glands have been lost. In contrast, minor ampullate glands often remain in taxa that have lost their cribellum (see, for example, Griswold et al. 2005; Murphy & Roberts 2015). We studied representatives of two groups that have lost the minor ampullate glands independently, in particular Hahniidae (*Antistea*, *Cybaeus*) and some Zodariidae (*Zodarion*, *Psammorygma*). However, the spigots of the minor ampullate glands have been observed in some other Zodariidae (Ramirez 2014).

In contrast to cribellate spiders, in the ecribellate spiders and *Dictyna* Sundevall, 1833 that are studied here the products of the major and minor ampullate glands presumably serve the same respective functions, i.e., minor ampullate fibers are mainly used for reinforcing the major ampullate fibers in draglines or in construction elements of the webs, such as in species studied by Work (1981) and Peters and Kovoor (1991). *Dictyna* produces cribellate capturing bands but they do not contain undulating fibers (Eberhard & Pereira 1993). Such undulating fibers are, however, found in some other dictynids, for example the genus *Mallos* O.P-Cambridge, 1902 (Griswold et al. 2005). It is thus not surprising that the morphology of these two gland types is almost identical in these spiders. In the family Agelenidae, this similarity also includes the presence of the system of three pairs of glands (i.e., three pairs of minor ampullate and three pairs of major ampullate glands), which, according to the findings of Townley et al. (1993) in *Araneus* Clerck, 1757, suggests that each of them function in different instars or at different times during the molt cycle (Townley et

al. 1993). The two accessory pairs produce fibers during alternate proecdyses (i.e., one pair producing fibers in odd-numbered stadia, the other pair producing fibers in even-numbered stadia).

The presence of non-functional pairs of glands is also manifested in spinneret morphology. There could be a tartipore – a sear on new cuticle after the collared opening – that allows the ducts of the functional secondary ampullate gland to remain attached to the spigot on the old exoskeleton during proecdysis (Townley et al. 1993). The spigot of the last open secondary gland is replaced by a so-called nubbin in adults, i.e., the stage where the secondary ampullate glands are not necessary any more (Townley et al. 1993). Moulting spiders do not capture prey, thus the absence of this system in the studied cribellate spiders, where the minor ampullate glands play a role in production of adhesive capturing bands, is not surprising. In contrast, in the Agelenidae the minor ampullate glands presumably play the same role as the major ampullate glands – their products might participate in draglines and shelters that are produced even during proecdysis.

Spiders of the family Agelenidae build relatively large horizontal sheet webs instead of producing cribellate bands in order to capture their prey. For such webs, they need a large amount of silk. Therefore, it might have been advantageous for them to retain minor ampullate glands that have greater numbers of branches (Fig. 2F), thereby creating a larger surface area of secretory epithelium than the simple gland structures. Some species producing remarkably large webs, for example the genus *Eratigena*, and primary glands of *Agelena* and *Allagelena*, appear to have further enlarged the secretory epithelium surface area through increasing the number of branches (Fig. 2H, J & K). These branches probably produce the same product (potentially in contrast to cribellate relatives).

In the other representatives of this family, branching is absent. In the species with simple glands that construct large webs (for example, some species of *Tegenaria*, and the genera *Agelenopsis* and *Histopona*), the secretory epithelium is enlarged by elongation (Fig. 2C–E).

Multiple branching of the secretory part of the minor and also the major ampullate glands in some Agelenidae is a unique feature among spider silk glands. Evolution of elongation instead of branching of the secretory part could have been driven by more advantageous rheology inside the simple glands. In order to decrease the energetic demands of spinning, spiders spin the fibers from the protein solution behaving as a liquid crystal (Knight & Vollrath 1999). The initially randomly oriented proteins aggregate end-to-end into rod-shaped units. Due to a very high concentration of the solution (up to 50% of proteins, Tillinghast et al. 1984), these rods interact by non-covalent forces. As a result, they orient themselves parallel to one another (Viney et al. 1994; Viney 1997). This phase flows as a liquid but maintains some molecular orientation of the crystal (Vollrath & Knight 2001). In branched glands, formation of the liquid crystal might be more complicated. From particular branches, the differently oriented protein molecules flow together. In contrast, in simple glands the formation of the liquid crystal is not disturbed by flows from different directions, and thus it is probably faster

Table 2.—The morphological features of the minor ampullate glands of studied spider species from representatives of the RTA clade and its outgroups. The term "variable" characterizing the length of branches means intraspecific variation in this character. x – the character is irrelevant in the species. 1° = primary, 2° = secondary. "Ampulla constriction" = constriction of ampulla close to duct funnel. "No. MiAG spigots" = Number of minor ampullate gland spigots in ♀, following Murphy & Roberts 2015, Miller et al. 2012. "No. undulating lines" follows Eberhard & Pereira 1993, Griswold et al. 2005.

Family/Species	Shape of tail	Length of branches	Direction of shorter branch	Ampulla	Ampulla constriction	No. MiAG spigots	No. undulating lines	Figure in Text
Cribellum present								
Amaurobiidae								
Amaurobius fenestralis	Bifurcated	Almost same	Toward spinneret	Inconspicuous	Absent	1	1	
Amaurobius ferox	Bifurcated	Almost same	Toward spinneret	Obvious	Present			1D E
Amaurobius jugorum	Bifurcated	Almost same	Toward spinneret	Inconspicuous	Present			1F
Callobius bennetti	Bifurcated	One significantly shorter	Toward spinneret	Absent	Absent			1G
Amphinectidae								
Metaltella simoni	Bifurcated	One significantly shorter	Toward spinneret	Absent	Absent		0–1	1C
Desidae								
Phryganoporus candidus	Bifurcated	Almost same	To the sides	Obvious	Present	1		1H
Paramatachia							0	
Badumna							1	
Dictynidae								
Dictyna arundinacea, uncinata	Unbranched	x	x	Absent	Absent	1		3D
Dictyna							0	
Mallos							1	
Eresidae								
Seothyra schreineri	Unbranched	x	x	Present	Inconspicuous	1–4		3A
Stegodyphus							3-4	
Phyxelididae						1	1	
Themacrys silvicola	Bifurcated	Almost same	Toward spinneret	Absent	Absent			1A
Vidole sothoana	Bifurcated	One significantly shorter	Toward spinneret	Absent	Absent			1B
Titanoecidae								
Titanoeca quadriguttata	Bifurcated	Almost same	To the front	Inconspicuous	Absent		1	1H
Titanoeca schineri	Bifurcated	Almost same	To the front	Inconspicuous	Absent			
Cribellum absent								
Agelenidae								
Agelena labyrinthica	1° pair irregularly branched, 2° pairs unbranched	Variable	x	Present	Absent	1–2	0	2J
Agelenopsis pennsylvanica	Unbranched	x	x	Obvious	Present			2L
Allagelena gracilens	1° pair irregularly branched, 2° pairs unbranched	Variable	x	Present	Absent			2K
Coelotes terrestris	Unbranched	x	x	Absent	Absent			2A
Eratigena agrestis	Irregularly branched	Variable	x	Obvious	Absent			2H
Eratigena atrica	Irregularly branched	Variable	x	Obvious	Absent			2I
Histopona torpida	Unbranched	x	x	Absent	Absent			2C
Tegenaria campestris	Unbranched	x	x	Absent	Absent			2D
Tegenaria domestica	Unbranched	x	x	Present	Present			2G
Tegenaria ferruginea	Irregularly branched	Variable	x	Absent	Inconspicuous			2F
Tegenaria silvestris	Unbranched	x	x	Present	Present			2E
Wadotes calcaratus	Unbranched	x	x	Absent	Absent	1	0	2B
Dictynidae								
Argyroneta aquatica	Unbranched	x	x	Absent	Absent		0	3E
Cicurinidae								

Table 2.—Continued.

Family/Species	Shape of tail	Length of branches	Direction of shorter branch	Ampulla	Ampulla constriction	No. MiAG spigots	No. undulating lines	Figure in Text
Cicurina cicur	Unbranched	x	x	Absent	Absent	0		3C
Cryphoeca						1		
Cicurina, Tuberta, Mastigusa							0	
Hersiliidae								
Hersiliola						1		
Hersilia						2		
Tyrotama australis	Unbranched	x	x	Absent	Present			3B

and more accurate. Therefore, in simple glands the spinning is perhaps energetically less demanding.

The species from the agelenid subfamily Coelotinae have only very small funnel webs. They possess relatively small minor ampullate glands that significantly differ from the minor ampullate glands of other studied species by very short distal and very long proximal secretory zone (Fig. 2A & B). Such glands probably produce fibers with a thin core and a thick outer coat. The ratio between the size of the proximal and the distal secretory zone resembles that of piriform glands that produce fibers covered by glue (Kovoor 1987). It is possible that the minor ampullate glands in the Coelotinae also produce adhesive fibers functionally substituting for cribellate capturing bands.

The mentioned conclusions are based only on our knowledge about the studied group of RTA spiders (Amaurobioidea in broad sense). Outside this group, the morphological and functional features of the minor ampullate glands are different. For example, cribellate members of the Eresidae from the very base of araneomorph spiders also possess undulating fibers in the adhesive capturing bands, but the minor ampullate glands are simple (Fig. 3A). Simple minor ampullate glands are also observed in other cribellate, uloborid spiders (Kovoor 1977; Kovoor & Peters 1988), which are known for their orb webs with a cribellate spiral instead of a spiral with viscous glue. However, in these cases their cribellate bands do not contain undulating fibers (Eberhard & Pereira 1993), and instead minor ampullate fibers are used, together with the major ampullate fibers, for frames of their orb webs (Peters 1983, 1984).

The phylogenetic relationships among the families at the base of the RTA clade are still very unclear. The difficulties with uncovering relationships of particular groups of this clade are caused by the conflict among characters (homoplasy) and the lack of informative synapomorphies. Many characters from spigot morphology, related to silk glands, have been already extensively used in phylogenetic analyses (for example, Griswold et al. 2005). However, internal silk gland morphology provides many more characters that might be informative for future phylogenetic analyses.

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Egg toxicity in diverse spider taxa

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Abstract. Eggs of black widow spiders in the genus *Latrodectus* Walckenaer, 1805 (Theridiidae) are known to be toxic when injected into mammals. We surveyed eggs from 39 species of spiders in 21 families to determine if spider egg toxicity is a unique property of widow spiders, or if spider egg toxicity is associated with other spider taxa. Eggs from 13 species of spiders in three families were determined to be highly lethal to mice, eggs from four species in four families were moderately lethal, and eggs from 22 species in 17 families lacked detectable lethality. Egg lethality appears to have evolved multiple times in spiders within the Araneidae, Theridiidae, and Agelenidae, and possibly also within the Tetragnathidae, Pimoidae, and Linyphiidae. These toxins in the various spiders may have differing chemical structures and could represent new sources of toxins that may be of future research interest.

Keywords: *Latrodectus, Steatoda, Araneus, Neoscona, Loxosceles*, toxin

Spiders are well known for their venomous bites and for the properties of their associated venoms. In contrast, potential toxins in non-venomous parts of spiders have received scant attention, much of it many years ago. In the late 1800s and early 1900s, R. Kobert, H. Sachs, V. Kellogg, R. Levy and others investigated the toxic properties of spider body tissues and eggs in several species of *Latrodectus* Walckenaer, 1805 for their ability to cause hemolysis and lethality in various animals including rabbits, guinea pigs, cats, dogs, and horses (Kellogg 1915; Bettini & Maroli 1978). D'Amour et al. (1936) extensively investigated the venom and egg toxicity of *Latrodectus* and reported that the precipitates of homogenized eggs, but not of young spiderlings, were toxic when injected intraperitoneally into rats. The authors also demonstrated that the heat-labile material in *Latrodectus* eggs was highly hemolytic to suspended rat erythrocytes.

Russell and associates revisited the properties of black widow (*L. hesperus* Chamberlin & Ivie, 1935) egg toxins and reported that eggs were highly lethal when injected intravenously into mice, and were hemorrhagic when injected intradermally into rabbits (Buffkin & Russell 1971, 1973). The effects from the injected black widow egg toxins were "dramatically different" than those elicited from *Latrodectus* venom toxins (Buffkin et al. 1971). Empty egg shells were not lethal (Buffkin & Russell 1971; Buffkin et al. 1971). They later partially purified several egg protein fractions and reported the main toxin to be a protein of 90–97 kDa (Buffkin et al. 1978). Several active toxins from eggs of *L. tredecimguttatus* (Rossi, 1790), a congeneric of *L. hesperus*, were subsequently isolated and shown to be unique and different from spider venom proteins or other known proteins (Li et al. 2013, 2014; Yan et al. 2014; Lei et al. 2015). One protein of molecular weight 23.8 kDa was highly toxic to mice. In contrast, the main venom component active against vertebrates is α-latrotoxin, which has a molecular weight of about 130 kDa (Rash & Hodgson 2002). Newly emerged widow spiderlings also exhibited high toxicity to mice (Peng et al. 2014).

Debated within the literature is the question of whether egg toxicity is limited to the genus *Latrodectus*, or if spider egg toxins are present in other taxa. Sachs (reported in Kellogg 1915) recorded a toxic principle in the body fluid of *Araneus diadematus* Clerck, 1757 (Araneidae), whereas the bite of that spider caused only locally irritating results. McKeown et al. (2014) also reported a clinical study involving five bites by *A. diadematus*, none of which resulted in serious injury or dermonecrotic lesions, though the bites sometimes caused minor to moderate reactions. D'Amour et al. (1936), working in Denver, Colorado, USA found that the eggs of "the common brown house spider" (species not given, though only one species in Denver, *Parasteatoda tepidariorum* (C.L. Koch, 1841) (Theridiidae), was referred to at that time as the "common house spider" (Paula Cushing, pers. comm.)) were toxic, though less so than those of *Latrodectus*. The authors felt that this poisonous material is found in the tissue fluids of all spiders, especially in their eggs. Buffkin et al. (1971) found no toxicity of eggs from the Arizona recluse spider, *Loxosceles arizonica* Gertsch & Mulaik, 1940 (Sicariidae), a medically important species, and *Araneus* sp. (Araneidae) when tested against mice. They did not explain their reasoning for choosing these two species and the eggs of "several other spider species" for their survey.

The purpose of the present report is to provide a clearer picture of the possible occurrence of egg toxins within a diversity of spider taxa via a broad survey of species. We sought to determine if egg toxins were restricted to *Latrodectus*, were present in close relatives to that genus, or were widespread throughout the Theridiidae, the large family that includes *Latrodectus*. Our goal was to determine if the evolution of spider egg toxins was a single event, or independently occurred several times within spider lineages. As a by-product of this research, we hope that these results might initiate investigations of spider eggs as a source of new toxins of potential use as pharmaceutical tools.

METHODS

Spiders.—All spiders were collected from natural environments in the states of California, Washington, Arizona and Kansas, all in the USA. The spiders were brought into the lab and fed *ad libitum* until they produced egg sacs. Egg sacs, or in some cases live pregnant spiders, were mailed to Tucson,

Table 1.—Taxa, lethality to mice of spider egg extracts, and collection locations of spiders used in this study. Taxa arranged alphabetically by family, then genus and species.

Spider species	Family	LD$_{50}$ (i.v., mg/kg)	Collection location
Agelenopsis potteri (Blackwall, 1846)	Agelenidae	>227.2	USA: WA: King Co.: Enumclaw
Eratigena agrestis (Walckenaer, 1802)	Agelenidae	9.3	USA: WA: King Co.: Enumclaw
E. atrica (C.L. Koch, 1843)	Agelenidae	9.3	USA: WA: King Co.: Enumclaw
Hololena nedra Chamberlin & Ivie, 1942	Agelenidae	>227.2	USA: CA: Fresno Co.: Fowler
Tegenaria domestica (Clerck, 1757)	Agelenidae	40.2	USA: WA: King Co.: Enumclaw
Callobius severus (Simon, 1884)	Amaurobiidae	>227.2	USA: WA: King Co.: Enumclaw
Metaltella simoni (Keyserling, 1878)	Amphinectidae	>227.2	USA: CA: Riverside Co.: Riverside
Araneus diadematus Clerck, 1757	Araneidae	2.5	USA: WA: King Co.: Enumclaw
Neoscona oaxacensis (Keyserling, 1863)	Araneidae	1.3	USA: CA: Fresno Co.: Fresno
Zygiella atrica (C.L. Koch, 1845)	Araneidae	5.0	USA: WA: King Co.: Enumclaw
Cicurina pusilla (Simon, 1886)	Dictynidae	>227.2	USA: WA: King Co.: Enumclaw
Cheiracanthium inclusum (Hentz, 1847)	Eutichuridae	>227.2	USA: CA: Riverside Co.: Riverside
Herpyllus propinquus (Keyserling, 1887)	Gnaphosidae	>227.2	USA: CA: Riverside Co.: Riverside
Urozelotes rusticus (L. Koch, 1872)	Gnaphosidae	>227.2	USA: CA: Riverside Co.: Riverside
Bathyphantes brevipes (Emerton, 1917)	Linyphiidae	80.3	USA: WA: King Co.: Enumclaw
Hogna carolinensis (Walckenaer, 1805)	Lycosidae	>227.2	USA: AZ: Cochise Co.: Willcox
Pardosa californica Keyserling, 1887	Lycosidae	>227.2	USA: CA: Riverside Co.: Riverside
Reo eutypus Chamberlin & Ivie, 1935	Mimetidae	>227.2	USA: CA: Riverside Co.: Riverside
Philodromus dispar Walckenaer, 1826	Philodromidae	>227.2	USA: WA: King Co.: Enumclaw
Pholcus phalangioides (Fuesslin, 1775)	Pholcidae	>227.2	USA: CA: Riverside Co.: Riverside
Pimoa altioculata (Keyserling, 1886)	Pimoidae	80.3	USA: WA: King Co.: Enumclaw
Salticus scenicus (Clerck, 1757)	Salticidae	>227.2	USA: WA: King Co.: Enumclaw
Scytodes globula Nicolet, 1849	Scytodidae	>227.2	USA: CA: Riverside Co.: Riverside
Loxosceles rechsa Gertsch & Mulaik, 1940	Sicariidae	>227.2	USA: KS: Johnson Co.: Lenexa
Olios gigauteus Keyserling, 1884	Sparassidae	>227.2	USA: AZ: Pima Co.: Tucson
Metellina segmentata (Clerck, 1757)	Tetragnathidae	40.2	USA: WA: King Co.: Enumclaw
Enoplognatha ovata (Clerck, 1757)	Theridiidae	5.0	USA: WA: King Co.: Enumclaw
E. thoracica (Hahn, 1833)	Theridiidae	5.1	USA: WA: King Co.: Enumclaw
Latrodectus geometricus C.L. Koch, 1841	Theridiidae	10.0	USA: CA: Riverside Co.: Riverside
L. hesperus Chamberlin & Ivie, 1935	Theridiidae	10.0	USA: CA: Riverside Co.: Riverside
Parasteatoda tepidariorum (C.L. Koch, 1841)	Theridiidae	>227.2	USA: CA: Riverside Co.: Riverside
Steatoda albomaculata (De Geer, 1778)	Theridiidae	14.2	USA: WA: King Co.: Enumclaw
S. bipunctata (Linnaeus, 1758)	Theridiidae	10.0	USA: WA: King Co.: Enumclaw
S. grossa (C.L. Koch, 1838)	Theridiidae	10.0	USA: CA: Riverside Co.: Riverside
S. triangulosa (Walckenaer, 1802)	Theridiidae	5.0	USA: CA: Riverside Co.: Riverside
Theridion melanurum Hahn, 1831	Theridiidae	>227.2	USA: CA: Riverside Co.: Riverside
Tidarren sisyphoides (Walckenaer, 1841)	Theridiidae	>227.2	USA: CA: Riverside Co.: Riverside
Trachelas mexicanus Banks, 1898	Trachelidae	>227.2	USA: AZ: Maricopa Co.: Phoenix
Uloborus diversus Marks, 1898	Uloboridae	>227.2	USA: CA: San Bernardino Co.: Redlands

Arizona, USA in insulated containers with cool packs. Intentional efforts were made to collect as broad a diversity of spiders from different taxa as possible. In total, 39 species in 33 genera and 21 families were analyzed (Table 1).

Processing of eggs.—The eggs were removed from their sacs using forceps and a dissecting microscope, weighed fresh, and frozen at −25° C until used. Eggs that were discolored, shrunken, or contained unhatched spiderlings were discarded.

Lethality determinations.—White ICR mice of mixed sex and weighing 20–25 g were used for lethality tests. Whole spider eggs were homogenized in saline (0.9% NaCl) and injected intravenously (i.v.) in volumes of 100 µl or less into tail veins of mice. Cohorts of four mice per dose were used and doses were doubled until an LD$_{50}$ value was reached, or the highest dose equaled 227.2 mg/kg. In some instances, only enough egg extract was available to test 2 or 3 mice at a dose level of 227.2 mg/kg, in which case, lethality was recorded as

>227.2 mg/kg. Lethality was determined at 24 h as described in Schmidt (1995).

RESULTS AND DISCUSSION

Eggs from three spider families were highly lethal to mice with LD$_{50}$ values of 1.3–14.2 mg/kg (Table 1). Four spider families contained members whose eggs were moderately toxic in the range of 15–81 mg/kg, and 17 spider families possessed at least some members with eggs having no detectable lethality at 227.2 mg/kg, the highest dose found practical to test. Overall, 17 of the 39 species, just under half of the tested species, produced toxic eggs. The most toxic eggs, with a lethality of 1.3 mg/kg of whole eggs, were produced by the orb-web-building spider *Neoscona oaxacensis*, a species whose bite produces only minor local reactions and is not considered toxic to humans. Three spider genera, including the widows (*Latrodectus*), false widows (*Steatoda* Sundevall, 1833), and

Enoplognatha Pavesi, 1880, all in the comb-footed spider family Theridiidae, contain species with toxic eggs, though none were as toxic as the eggs of *Neoscona oaxacensis*. Two or more species from within a genus were tested in four genera, three within the Theridiidae and one within the Agelenidae. In all cases, the lethalities of eggs from species within a single genus were either the same, or similar, in being very toxic.

Theridiidae.—Particular emphasis was placed on the eggs of *Latrodectus* species because they were the first species to be noted to have toxic eggs. Eggs of the two species analyzed here, plus of the Old World species *L. tredecimguttatus* (Li et al. 2013) are all toxic, an indication that egg toxicity within the widow genus is widespread, if not universal. The sister genus to *Latrodectus* is *Steatoda*, the false widows (Agnarsson 2004; Arnedo et al. 2004), also known to possess at least mildly toxic venoms similar to those of the true widows (Isbister & Gray 2003). For this reason, we tested four species of *Steatoda*, all of which have egg toxicities similar to those of *Latrodectus*. The Theridiidae is an enormously diverse family of over 120 genera and 2400 species, the fourth largest of the spider families worldwide, and a family whose phylogeny is well-established (Agnarsson 2004; Arnedo et al. 2004). We tested members of six theridiid genera and found three genera to have toxic eggs, and three genera with non-toxic eggs. The third toxic genus, *Enoplognatha*, is not closely related to the subfamily Latrodectinae, in which *Latrodectus* and *Steatoda* reside. The other three genera, *Theridion* Walckenaer, 1805, *Parasteatoda* Archer, 1946 and *Tidarren* Chamberlin & Ivie, 1934, all lack egg toxicity and are in the distant subfamily Theridiinae. From these data, we conclude that egg toxicity evolved independently at least once, possibly twice, in the Theridiidae, but is not universal within the family. Because we were not able to analyze eggs from any genus between the latrodectines and *Enoplognatha*, we cannot determine if toxicity of eggs evolved once or twice. Likewise, we cannot determine if an ancestor of the latrodectines and *Enoplognatha* had toxic eggs and whether that toxicity was lost before the Theridiinae lineage, or if it evolved independently twice, once in the latrodectines and again in a lineage leading to *Enoplognatha*.

Araneidae.—Three species in different genera were evaluated. The eggs of *Neoscona oaxacensis*, a species whose bite is not considered toxic to humans, were the most lethal of all spider eggs. The two other orb-web builders, *Araneus diadematus* and *Zygiella atrica*, also produced highly lethal eggs, though somewhat less lethal than those of *N. oaxacensis*.

Agelenidae.—Five species in four genera in the Agelenidae were analyzed, with both species in the genus *Eratigena* Bolzern, Burckhardt & Hänggi, 2013, exhibiting high egg toxicity. In contrast, eggs of *Tegenaria domestica* exhibited moderate toxicity, and eggs of both *Agelenopsis potteri* and *Hololena nedra*, were non-toxic (Table 1). Recently, the two agelenid species with highly toxic eggs, the hobo spider *Eratigena agrestis* and its congener *E. atrica*, have been transferred to a newly erected genus from the closely related genus *Tegenaria* Latreille, 1804. That eggs of *T. domestica* were only moderately toxic compared to *Eratigena* corroborates the taxonomic transfer by Bolzern et al. (2013).

Other spider families.—Based on the phylogeny of Nentwig (2013), the families Pholcidae, Sicariidae and Scytodidae are

nearer the base of the spider tree than are the toxic species. The members of these families that we tested all had non-toxic eggs. Within the Orbiculariae, *Uloborus diversus* eggs were also non-toxic. Moving up the Orbiculariae phylogeny is the Araneoidea, which contains the families Theridiidae, Mimetidae, Tetragnathidae, Araneidae, Pimoidae, and Linyphiidae. We tested members of all six of these families and all, except the Mimetidae, contained species with toxic eggs.

The sister clade to the Orbiculariae spiders is the RTA clade. We tested members of 11 families within this clade and found only those in the Agelenidae contained members with toxic eggs. The Agelenidae reside midway within the RTA clade. These findings within the Agelenidae suggest that egg toxicity evolved independently at least once within the RTA branch and not in the ancestor of that clade.

Comparisons with other studies.—Direct comparisons of our lethality results to those in the literature are difficult. Kellogg (1915) did not provide quantitative data based upon toxin weight and D'Amour et al. (1936) used number of eggs tested. Buffkin et al. (1971) and Li et al. (2013) used either lyophilized egg extracts, or partially purified extracts of eggs. In our tests, we used fresh frozen whole eggs without drying or processing beyond homogenization to minimize possibilities of toxin degradation. These techniques also mean that the actual egg toxins were diluted and are considerably more toxic than reported in our data. Our results differ from those of the other investigators in the taxonomic scope of the spider eggs investigated. D'Amour et al. (1936) felt that body and egg toxins likely were present throughout spiders. In contrast, Buffkin et al. (1971) indicated a lack of egg toxicity in the several species tested. Our results reveal about half of our tested spiders produced toxic eggs, thereby indicating egg toxicity is not limited to *Latrodectus*. The cross spider, *Araneus diadematus*, represents an interesting case study. As reported in Kellogg (1915), both Sachs and Koch found non-venom tissues of *A. diadematus* to contain toxins whereas Buffkin et al. (1971) reported eggs of *Araneus* sp. (presumably *A. diadematus* based on their later work (Russell & Maretić 1979)) to be non-lethal. We found eggs of *A. diadematus* to be highly lethal, four times more lethal than *Latrodectus* eggs.

Biological role of egg toxins.—Our lack of understanding of the biological function of toxins in spider eggs is a source of personal frustration to the authors. This, however, is not without precedent among spider toxins. For example, humans and primates are highly susceptible to the effects of venom toxins from the Sydney funnel web spider, *Atrax robustus* Pickard-Cambridge, 1877 (Hexathelidae), whereas non-primates, including rodents and domestic animals such as dogs are little affected (Sutherland & Tibballs 2001). Since humans and primates were absent until geologically recent times, the evolution of Sydney funnel web spider toxins was unlikely in response to humans or primates.

The fact that egg toxicity is widely distributed within spiders, likely having independently evolved multiple times begs that these toxins have some important biological function. Protection from predators and parasites is one possible role. Arguing against a defensive role are the observations that *Latrodectus* eggs fed to mice produced no symptoms of toxicity (Buffkin et al. 1971) and that *Latrodectus*, *Steatoda*, and *Araneus* Clerck, 1757 have numerous specialized wasp parasitoids and a

chloropid fly egg predator that attack their eggs (Austin 1985; Vetter et al. 2012). In addition, one of us (R.S.V.) fed large gravid female western black widows, *Latrodectus hesperus*, to several potential predators including mice, rats, scorpions, tarantulas, centipedes, and alligator lizards. These animals readily ate the spiders without deleterious effects. An interesting exception was a shrew, *Sorex* sp., that exhibited a negative reaction upon piercing an egg sac with its teeth and immediately retreated and rubbed its snout in the soil of the cage. Arguing in favor of egg toxins having predator protective potential are the observations of Russell & Maretić (1979) that showed lethality and altered behavior in *A. diadematus* when they were fed *Latrodectus* egg extracts, and the shrew behavior described above. The role of spider egg toxins might turn out to be selective protection against some predators or parasitoids, but not others.

Conclusions.—Spider egg toxicity appears to have evolved multiple times: once or twice within the Theridiidae, once in the Tetragnathidae, at least once in the clade containing Araneidae, Pimoidae, Linyphiidae (plus others), and at least once in the Agelenidae. Egg toxicity is not correlated with venom toxicity, as only *Latrodectus* has both eggs and venom that are toxic to mammals. The fact that many different spider taxa produce toxic eggs suggests that spider egg toxins constitute a diverse group of new toxins, or even possibly new classes of toxins, that might be potentially useful in various areas of toxicology or pharmacology.

ACKNOWLEDGMENTS

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Abiotic factors and biotic interactions jointly drive spider assemblages in nest-boxes in mixed forests

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Abstract. Although spiders are common inhabitants of tree cavities, factors that drive their community structure in these microhabitats are little known. Here we investigated whether bark type, season, intraguild predation (IGP) among spiders, and presence of vertebrate predators can influence the spider community structure in tree cavities. We examined spider abundance and the taxonomic and functional composition of spiders in nest-boxes within two mixed forest stands in central Slovakia in 2012–2013. In total, 1211 spiders belonging to 31 species were sampled from 60 nest-boxes at two sites over three seasons. Spider abundance peaked in autumn as spiders sought wintering sites. Guilds and taxonomic composition changed seasonally with spring and autumn communities dominated by "Other hunters" (Anyphaenidae, Clubionidae, Philodromidae) while during summer the community was dominated by "Sheet web weavers" (Linyphiidae). The guild and taxonomic turnover may be partly explained by the interaction between spiders' phenology and IGP exerted by winter-active spiders on smaller spiders from autumn until spring. Bark type influenced the guild composition as dominance of "Space web weavers" was higher in trees with rough bark than in trees with smooth bark. The rough bark also reduced the intensity of IGP by *Anyphaena accentuata* (Sundevall, 1833) on philodromids. The presence of insectivorous birds reduced the abundance of spiders by 67%. The presence of bird predators altered the guild composition as they affected mostly the web spiders. The results show that the biotic interactions and abiotic factors interactively determined the spider community structure in the nest-boxes depending on spiders' functional traits.

Keywords: Guilds, intraguild predation, predation, spiders, birds, cavities

Tree cavities are crucial microhabitats for a wide variety of animals in temperate and boreal forests. Cavities play an important role as a keystone vegetation structure and component in biodiversity conservation (Tews et al. 2004; Regnery et al. 2013). Cavities provide breeding, feeding and roosting habitat for hole-nesting birds (Bai & Mühlenberg 2008; Mainwaring 2011; Robles et al. 2011), small mammals (Czeszczewik et al. 2008; Regnery et al. 2013), social hymenopterans (Morato & Martins 2006; Broughton et al. 2015), earwigs (Burnip at al. 2002) and saproxylic beetles (Buse et al. 2007; Jansson et al. 2009; Sverdrup-Thygeson et al. 2010; Sebek et al. 2013).

The density of cavities depends on tree species composition in an area and increases with the age of forest stands (Larrieu & Cabanettes 2012). However, most cavity users (except certain primary cavity nesting birds) are not able to build their own cavities, and are, therefore, strongly limited by the availability of existing holes. In primeval forests, this limitation is reduced by a surplus of tree-holes with high structural variability (Wesołowski 2007) because of an absence of management practices. Most European forests have changed from old mixed forests to young monocultures of single-aged plantations due to human management (Bradshaw 2004; Gamfeldt et al. 2013) and nest-boxes are often used to enhance bird and small mammal breeding possibilities and abundance (Twedt & Henne-Kerr 2001; Czeszczewik et al. 2008). Furthermore, nest-boxes are frequently used as a method to study the life histories of inhabitants of natural cavities because natural tree cavities can be difficult to examine. Few studies have evaluated the benefits of bird nest-boxes for invertebrates (e.g., hymenopterans; Stanback et al. 2009; Langowska et al. 2010).

Spiders are the most dominant of invertebrate predators occurring on and under bark, in bark crevices as well as in tree cavities, and they play an important role in food webs of forests (Růžička et al. 1991; Lawrence & Wise 2004; Szinetár & Horváth 2006). Spiders depend on vegetation structures because they use tree microhabitats for hunting prey, feeding, roosting, overwintering, mating, etc. (Wunderlich 1982; Boyed & Reeves 2003; Horváth et al. 2005).

However, current knowledge about ecological factors which can influence the community structure of spiders in tree cavities is limited to few studies of nest-boxes (Conner et al. 1995; Naeem et al. 2010). For example, McComb & Noble (1982) found that spider assemblages in nest-boxes varied seasonally, and Naeem et al. (2010) tested effect of habitat and composition of nest contents on spider abundance.

Spider assemblages are controlled by environmental factors (e.g., humidity, temperature, habitat structure) and by biotic interactions (e.g., competition, intraguild predation, predation) (Wise 1993; Samu et al. 1999; Gunnarson 2007; Gan et al. 2015; Mammola et al. 2016; Petcharad et al. 2016). In forests, spiders are influenced by the structure of ground vegetation and tree crown canopies. It has been shown that structural components in different forests types composed of different tree species support different spider assemblages (Gunnarsson 1990; Pinzón & Spence 2010; Korenko et al. 2011; Samu et al. 2014; Košulič et al. 2016; Petcharad et al. 2016). Bark type and structure can also affect spider species richness as was demonstrated by Nicolai (1986) who found fewer species on smooth bark than on fissured, scaly bark in temperate forests.

Temporal scale patterns also have a strong impact on spider assemblages. In the temperate zone of Central Europe, there are significant seasonal variations in temperature. The

colonisation and re-colonisation of tree trunks after winter onset can be described as a seasonal dynamic for arboreal spiders (Hsieh & Linsenmair 2012). For example, the abundance of spiders in artificial shelters in apple trees is higher in autumn and winter and lower in spring and summer (Isaia et al. 2010).

Spider assemblages in tree cavities can be affected also by predation. Birds frequently use tree cavities for breeding and roosting and several foliage gleaning and bark foraging species are important predators of spiders (Gajdoš & Krištín 1997; Naef-Daenzer et al. 2000). Birds can effectively reduce spider abundance and diversity (Askenmo et al. 1977; Gunnarsson 2007; Mestre et al. 2013; Gunnarsson & Wiklander 2015). Birds are visually oriented predators; spiders make use of passive defenses to limit their detection and predation by birds (Pekár 2014). Bird predation can also influence the composition of spider guilds. Gunnarson and Wiklander (2015) found that actively hunting spiders are more prone to bird predation than web weavers while Mestre et al. (2013) found an opposite pattern. Hence, birds are not only able to influence the abundance and diversity of spiders, but they may also represent a selective pressure which structures the species composition of spider assemblages based on spiders' primary defense mechanisms and guild affinition.

Another biotic interaction that strongly shapes spider communities is intraguild predation among spiders (IGP) (Wise 1993). Although araneophagy is pronounced mainly in the hunting spiders (Michalko & Pekár 2016), they prey not only on other hunting spiders but also on web spiders such as theridiids and linyphiids (Gunnarsson 1985; Finke & Denno 2006; Korenko & Pekár 2010; Michalko & Pekár 2015). The top predator among spiders therefore will be a hunting spider but the impact of IGP might be mainly size-dependent in the tree cavities (Gunnarsson 1985; Korenko & Pekár 2010; Michalko & Pekár 2015).

In this study, we investigated how season, bark type (rough vs. smooth), presence of birds, and IGP among spiders influence the abundance, taxonomic and guild composition of spider assemblages in artificial tree-cavities. We conducted an exclosure experiment with guarded nest-boxes to test if the presence of vertebrate predators can negatively influence spiders inhabiting tree cavities. We analysed spider guilds and primary defences in relation to the presence or absence of birds using nest-boxes.

METHODS

Study area and sampling.—The study was performed in two managed forest habitats 7.5 km apart in the southeastern part of the Kremnické vrchy Mountains in central Slovakia. The first site represented a mature mixed 90-year old oak-hornbeam forest (Stráže, 48° 34′ 41″ N, 19° 5′ 35″ E, 320–380 m asl., hornbeam 48%, oak 46%). The density of natural tree hollows at this site was 28.7/ha and density of bird boxes was 1.4/ha. The second site is a 113 year-old beech-fir forest stand (Kováčová, 48° 38′ 12″ N, 19° 4′ 59″ E, 480–530 m asl., fir 42%, beech 40%); both areas have been a part of long term studies of bird roosting and foraging behavior (Krištín 2002; Veľký et al. 2010). The density of natural tree hollows on the plot is 22.9/ha and bird box density 1.3/ha.

These study sites are in a moderately warm region with a mean annual temperature of about 6.8° C (Barna & Schieber 2011). Both study plots are under active forest management. In each plot, we placed 30 wood nest-boxes (270 × 125 × 125 mm, inlet 33 mm) on trees two meters above the ground with southern orientation in 2011. Boxes were arranged 50–70 m apart in a straight line. All nest-boxes were checked during April, June and October in 2012.

To investigate the influence of spider predators (birds and dormice) on spiders, we conducted an exclosure experiment. In March 2013, for half of the nest-boxes (n = 15 per forest stand) we fenced entrances to prevent access of vertebrate predators. Nest-boxes were then treated as follows: the first nest-box was open while the following 2 nest-boxes were closed, 4 open, 3 closed, 3 open, 3 closed, 3 open, 3 closed, 4 open, 4 closed. Nest-boxes were checked twice (May 9, 2013 and July 3, 2013).

Data collection.—Spiders were collected from nest-boxes with an exhaustor (aspirator), by beating the roofs of nest-boxes outside the nest-box on a white sheet (while the box was covered by a reserve roof in order to minimize disturbance of breeding birds) and then by manually collecting from crevices in the interior. Spiders were then preserved in 95% alcohol for later identification. All specimens were identified to species whenever possible following Roberts (1996) and Heimer & Nentwig (1991) using the nomenclature in the World Spider Catalog (2016) Version 17.0. Voucher specimens were deposited in the Arachnid collection at the Institute of Forest Ecology SAS in Zvolen, Slovakia.

Season and bark type.—The analyses were performed with R (R Development Core Team, 2015) and Canoco for Windows 5 (ter Braak & Šmilauer 2012). We evaluated bark type visually according to bark structure and tree species as follows: "rough bark" being oak (*Quercus robur*) and fir (*Abies alba*); and "smooth bark" being beech (*Fagus sylvatica*), linden (*Tilia* sp.) and hornbeam (*Carpinus betulus*). There were 10 trees with rough bark and 20 with smooth bark in the first study site and 16 trees with rough bark and 14 with smooth bark in the second study site. We analysed the effect of bark type (i.e., rough, smooth) and season (i.e., spring, summer, autumn) on the abundances of spiders using Linear Mixed Effects Models (LME) and using the R package "nlme" as the data were potentially autocorrelated at several levels (Pinheiro et al. 2015). The first source of autocorrelation may occur from measurements performed in the two localities. The second source of autocorrelation could be tree species since unmeasured species-specific traits besides bark roughness can influence the abundances of spiders. We did not treat tree species as a fixed effect because we were unable to separate the confounding effect of locality as different tree species were sampled at the two localities. The third possible source of autocorrelation was the repeated measurements performed on individual trees. Values for spider abundances were log (x+1) transformed to approach normal distribution of errors (Pekár & Brabec 2012). The fixed effects of LME were represented by bark type and season, and their interaction. Locality, tree species and identity of tree represented the nested random effects in the initial model. We determined the structure of random effects by removing the random effects and comparing competing models with AIC (Pekár & Brabec 2012). Tree species with six levels was then the only random effect in the

final model. We did not consider species richness of spiders because abundances and species richness were highly redundant.

We investigated the effects of bark type and season on community composition at the family level. We studied how bark type and season influenced the taxonomic compositions of the spider community by Canonical Correspondence Analysis (CCA). We used CCA as the preliminary Detrended Canonical Analysis (DCA) indicated a unimodal response by the length of the first axis being 4.6 (Šmilauer & Lepš 2014). Families with fewer than three individuals were excluded from the analysis as CCA is sensitive to the presence of rare species (Šmilauer & Lepš 2014). First, we performed a global test of significance for explanatory variables (i.e., season, bark type) to avoid Type I errors resulting from multiple comparisons (Šmilauer & Lepš 2014). We then performed forward selection to investigate the significance of particular variables. Significance was tested by Monte Carlo permutation tests restricted within the localities (Šmilauer & Lepš 2014).

To investigate the effect of bark type and season on the guild composition of the spider community, we used the categories proposed by Cardoso et al. (2011) as they are connected to the trophic niches of spiders (Michalko & Pekár 2016) and so they can be considered as guilds (Root 1967). The guilds are: Other hunters, Ground hunters, Ambushers, Orb web weavers, Space web weavers, Sheet web weavers, Sensing web weavers, and Specialists (Cardoso et al. 2011). We employed univariate methods as we investigated the effect of only one functional trait, i.e., guild (Šmilauer & Lepš 2014). For each nest-box with at least one spider, we computed proportions of each guild. For statistical evaluation we used Generalized Estimating Equations (GEE) as an extension of Generalized Linear Models (GLM) for autocorrelated data (Pekár & Brabec 2012) using the R package "geepack" (Højsgaard et al. 2006). As the response variable was proportion, we used GEE with binomial error structure and logit link (GEE-b). The explanatory variables were guild, season, bark type and two-fold interactions of guild with season and of guild with bark type. Therefore, the linear predictor was of multifactorial ANOVA type. Here, we used the tree ID as the grouping variable. We opted for "exchangeable" correlation structure as the number of measurements per group were low (≤3; Pekár & Brabec 2012).

Predator effect.—Birds, as important predators of spiders, were the most abundant predators (98%) in the nest-boxes. However, rodents that constituted the rest of the potential predators (2%) can also prey on arthropods including spiders (Butet & Delettre 2011). To study the influence of predator presence on the abundance of spiders, we pooled all vertebrate predator species, which could be affected by our exclosure experiments (i.e., birds: *Ficedula albicollis* Temminck, 1815, *Parus major* Linnaeus, 1758, *Cyanistes caeruleus* Linnaeus, 1758, *Sitta europaea* Linnaeus, 1758; dormice: *Muscardinus avellanarius* Linnaeus, 1758, and *Glis glis* Linnaeus, 1766). These four bird species bred in all unfenced nest-boxes in both study sites during the exclosure experiment in following numbers: *F. albicollis* in 60% of nest-boxes (18/30), *P. major* in 26.7% (8/30) and *C. caeruleus* and *S. europaea*, in 6.7% each (2/30) and two dormice species we found each only in one nest-box check before the start of birds' breeding. So, we

compared the abundance of spiders between unprotected and protected (fenced) nest-boxes by LME as the data were autocorrelated. Data were log (x+1) transformed to approach normal distribution of errors (Pekár & Brabec 2012). The presence of predators acted as a fixed variable while locality and tree ID acted as nested random variables.

To compare the community composition with respect to the taxonomy, partial CCA with season and locality as covariates was used, as the initial DCA showed long environmental gradient (SD = 5.7). To compare the guild composition of spiders in nest-boxes that were protected and unprotected from predators, we used the classifications proposed by Cardoso et al. (2011) but we combined "Ground" and "Other hunters" and "Specialists" into "Hunters" because the way of movement and/or morphology of spiders should be more important for birds than their trophic niche. We also investigated the differences in composition of primary defense mechanisms of spiders. We classified primary defense mechanisms of each species according to data reviewed by Pekár (2014), i.e., anachoresis, crypsis, masquerade, aposematism and Batesian mimicry. The primary defense mechanisms of those species that were not included in the review of Pekár (2014) were derived according to the prevalence of defense mechanism found among their congeners. Any species which could not be categorized were omitted from analysis (n = 5). To investigate the effect of predator presence on the functional composition of spider communities, we computed the proportion of each guild / defense mechanism per nest-box. We compared the proportions of guilds and primary defenses between nest-boxes protected from predators and those unprotected using Generalized Mixed Effect Models with binomial error structure and logit link (GLMM-b) within the R package "glmmADMB" (Skaug et al. 2014). The guilds / defense mechanism, predator presence and their interaction acted as fixed variables and the linear predictor was of multifactorial ANOVA type. Tree ID acted as the random variable in the final model (Zuur et al. 2009). There was no need for correction of overdispersion.

Intraguild predation.—To investigate the possible effect of IGP on the spider community structure we used only the data for *Anyphaena accentuata* (Sundevall, 1833), *Philodromus* Walckenaer, 1826 spp., and Theridiidae in autumn as only these reached sufficient abundances for a reasonable statistical inference. In addition, IGP is well documented among these spiders (Michalko & Pekár 2015; Petráková et al. 2016). *Anyphaena accentuata* acts as a top predator in this system in autumn and can affect philodromids and theridiids (Pekár et al. 2015). During autumn, philodromids are larger than theridiids, which represent a substantial part of their diet (Gunnarsson 1985; Michalko & Pekár 2015). Therefore, we explored the effect of *A. accentuata* on theridiids and philodromids and the effect of philodromids on theridiids. Although clubionids also reached sufficient abundances, we did not include them as the biologically uninterpretable patterns or large misfits occurred in the models. We used GEE with Poisson error structure (GEE-p) as the response variable was counts and the data were autocorrelated (Pekár & Brabec 2012). Tree species represented the block variable and we used "exchangeable" correlation structure. The linear predictor was of ANCOVA type. The bark type acted as a
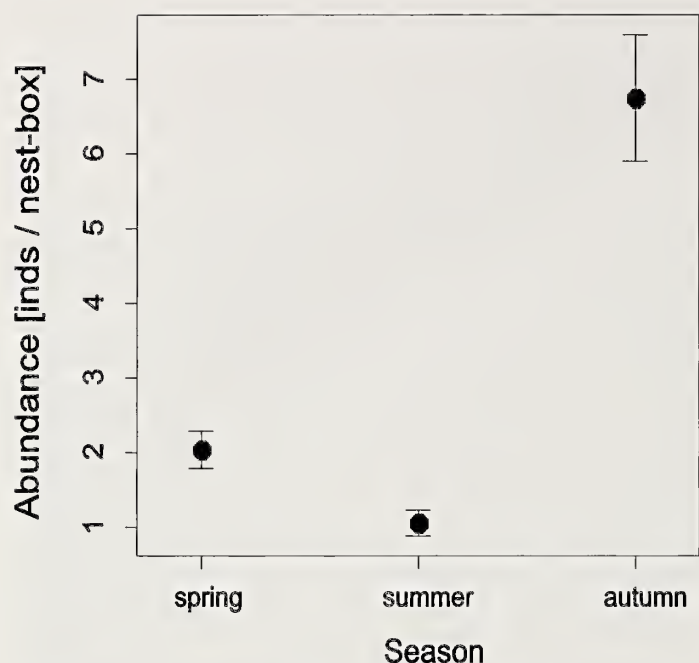
Figure 1.—Comparison of spider abundance in nest-boxes by vegetation season. Points and line segments show medians and SE, respectively; inds = individuals.

Figure 2.—CCA ordination diagram of spider families in relation to season.

factor whilst the abundance of the larger spider species acted as a covariate. We included also a quadratic form of the covariate because a hump-shaped relationship can arise. For example, both predators can respond positively at first (e.g., to prey availability or suitable environmental conditions), but as the interference intensifies the top predator starts to exclude the mesopredator (Holt & Polis 1997; Amarasekare 2008; Schmidt & Rypstra 2010; Schmidt et al. 2014). As the bark type can alter the intensity of IGP at the tree level, which can consequently translate in community composition in nest-boxes (Samu et al. 1999; Korenko & Pekár 2010), we included the interactions between bark and linear as well as quadratic form of the covariate. We employed Bonferroni adjustment of P-values to account for the multiple comparisons.

RESULTS

Spiders and birds were the most frequent inhabitants of the nest-boxes. Moths, earwigs, hymenopterans and small mammals were present in lower numbers, mostly outside the birds breeding season. In five nest-boxes, we found *Vespa crabro* nests (Hymenoptera). At both study sites, there were earwigs, *Chelidurella acanthopygia* (Géné, 1832) (Dermaptera) ($n = 73$), and *Lymantria* sp. (Lepidoptera) ($n = 112$). Two dormice species were found there: *Glis glis* ($n = 11$) and *Muscardinus avellanarius* ($n = 4$). Altogether five bird species were breeding and roosting in nest-boxes during both years; the most frequent and abundant bird species was *P. major* (41.9%, $n = 86$ occupied nest-boxes), followed by *F. albicollis* (37.2%), *S. europaea* (11.6%), and *C. caeruleus* and *Parus ater* (4.7% each). Altogether 633 spiders of 31 species from 13 families (from 1211 total specimens) were identified to species level (Appendix 1). Eight of these 31 species are exclusive bark-dwellers (i.e., habitat specialists), 16 are facultative bark dwellers and
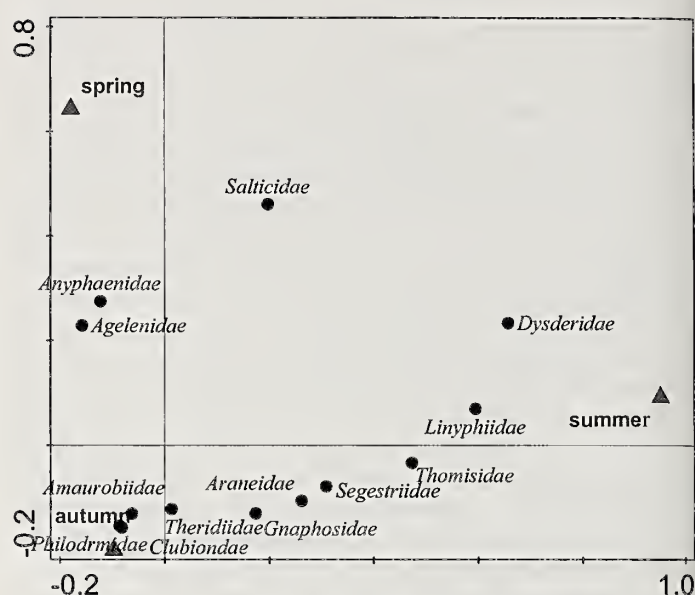
seven occur on the bark only accidentally (i.e., habitat generalists).

The two most abundant species were the arboreal and facultative bark dwelling *Anyphaena accentuata* which comprised 25% of all individuals, and the *Clubiona* Latreille, 1804 species complex (30%). Less abundant among our samples were *Platnickina tincta* (Walckenaer, 1802) with 9% and the *Philodromus aureolus* group (8%). The species *Arboricaria subopaca* Westring, 1861, also present in our samples, is listed as Vulnerable in the Red List of spiders of Slovakia (Gajdoš & Svatoň 2001).

Season and bark type.—The abundances of spiders significantly differed among seasons (LME, $F_{2,159} = 65.2$, $P < 0.001$, Fig. 1). Greatest abundances were in autumn (treatment contrasts, $P < 0.001$), followed by spring (treatment contrasts, $P < 0.002$), and summer. Bark type revealed no influence on overall spider abundances (LME, $F_{1,4} < 0.1$, $P = 0.97$).

Also community composition at the family level differed significantly only among seasons (CCA, pseudo-F = 12.3, $P = 0.001$; $R^2_{adj} = 0.14$, Fig. 2) and not between bark type (CCA, pseudo-F = 0.8, $P = 0.570$).

Season (GEE-b, $\chi^2_7 = 2120.8$, $P < 0.001$, Fig. 3A) as well as bark type (GEE-b, $\chi^2_{14} = 7348.4$, $P < 0.001$, Fig. 3B) influenced the functional community composition. In spring, the dominant guild was Other hunters, in summer Sheet web weavers, and in autumn Other hunters. The dominant guild on both bark types were Other hunters. The differences between the bark types was in the less dominant guilds, mainly Space web weavers (Fig. 3B).

Effects of predators.—The presence of predators significantly lowered the abundance of spiders in nest-boxes by 67% (LME, $F_{1,57} = 16.7$, $P < 0.001$, Fig. 4). There was no significant effect of the presence of predators on community composition of spiders at the family level (CCA, pseudo-F = 1.0, $P = 0.41$). However, the presence of predators influenced the functional composition of spider communities (GLMM-b, $\chi^2_4 = 10.6$, $P = 0.031$, Fig. 5). When predators were absent, the community was
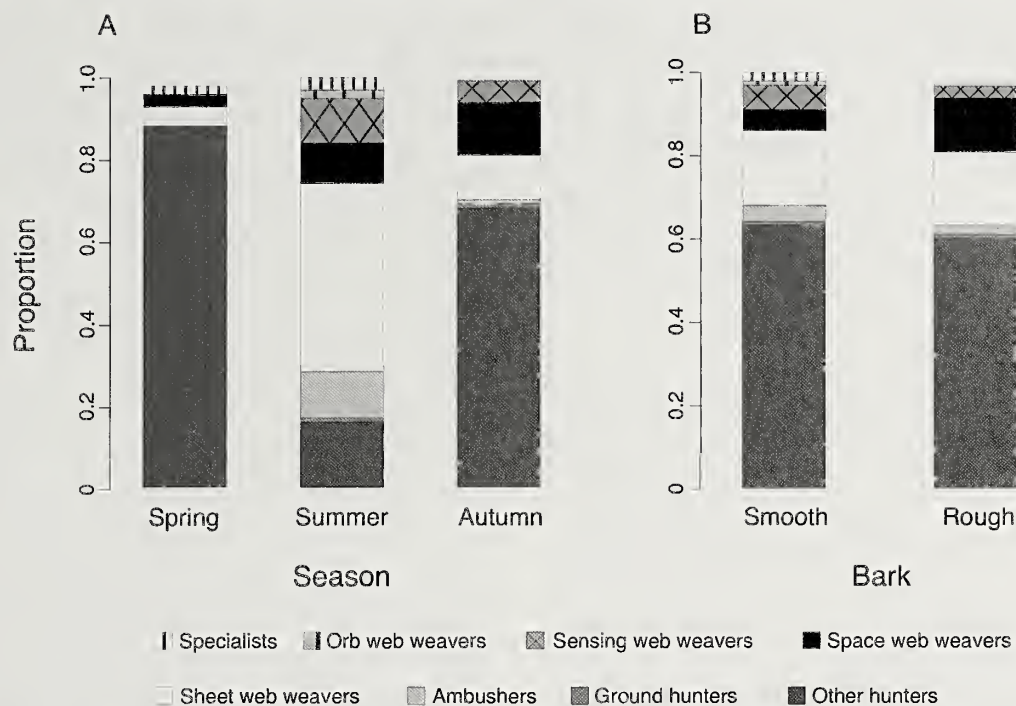
Figure 3.—Guild composition of spider communities in nest-boxes in relation to season (A) and bark type (B).

dominated by Sheet web weavers. When predators were present, the spider community was dominated by Hunters. However, there was no significant effect of predator presence on the composition of primary defense mechanisms in spider communities (GLMM-b, $\chi^2_2 = 0.3$, $P = 0.86$).
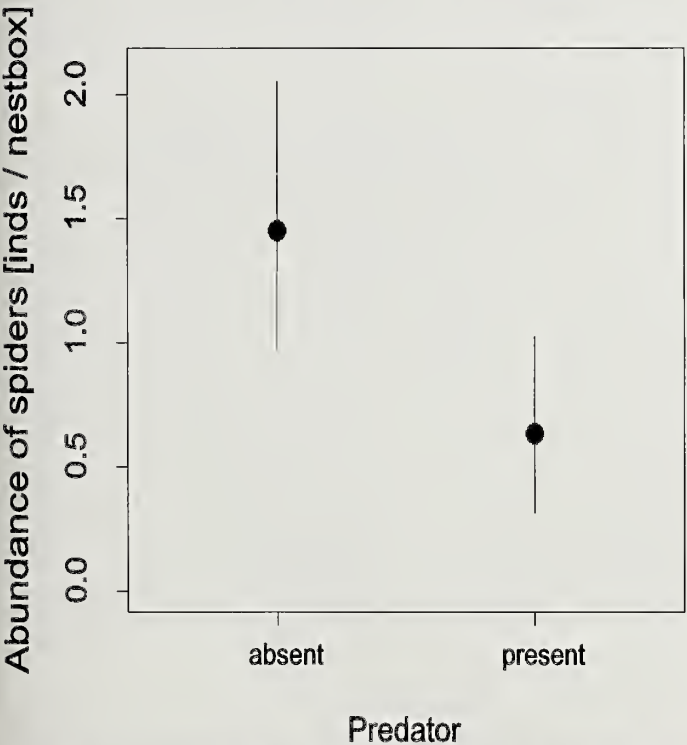
Intraguild predation.—There was a hump-shaped relationship between philodromids and *A. accentuata* (GEE-p, quadratic term, $\chi^2_1 = 9.3$, $P_{Bonferroni} = 0.006$, Fig. 6). The relationship differed between the bark types (GEE-p, interaction bark type: *Anyphaena*, $\chi^2_1 = 11.1$, $P_{Bonferroni} = 0.003$, Fig. 6). Consequently, in the smooth bark, the hump was not distinct as in the rough bark (Fig. 6). There was no significant

Figure 4.—Comparison of spider abundance in protected and unprotected nest-boxes. Points are medians and lines 95% CI; inds = individuals.
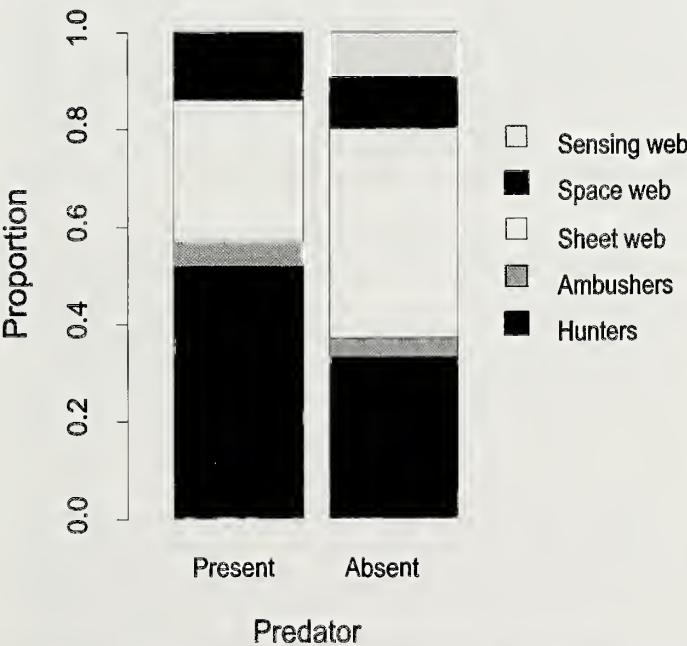
Figure 5.—Comparison of guild compositions of spider communities in protected and unprotected nest-boxes.
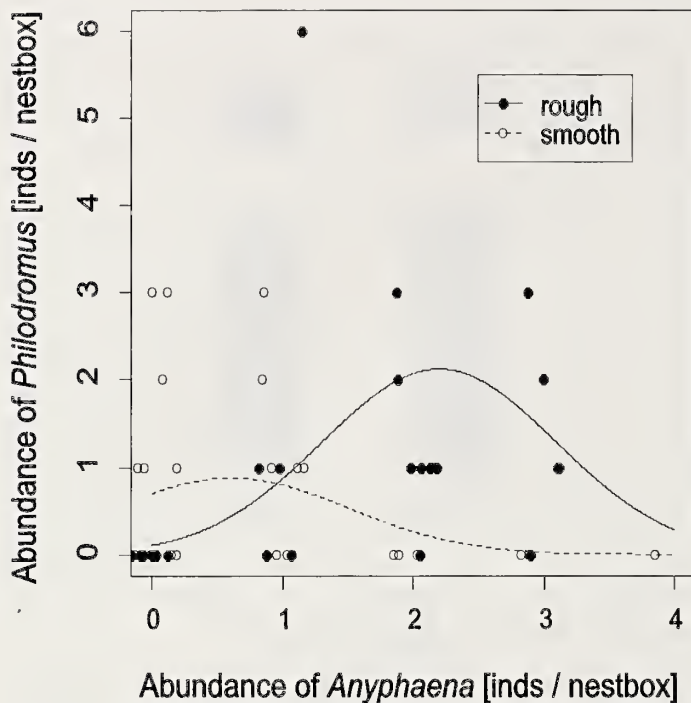
Figure 6.—Relationship between *Anyphaena accentuata* and *Philodromus* spp. abundances in nest-boxes in trees with different bark type. Small noise along the x-axis was added to show the data structure; inds = individuals.

effect of philodromids (GEE-p, $\chi^2_1 = 0.3$, $P = 0.560$) or *Anyphaena* (GEE-p, $\chi^2_1 = 0.1$, $P = 0.740$) on theridiids.

DISCUSSION

We analyzed how season, bark type, presence of predators, and IGP among spiders influence the abundance and composition of spider assemblages in artificial tree cavities. Spiders were the most common macro-invertebrates occurring in nest-boxes as was also found by Conner et al. (1995). Spiders that occupied nest-boxes were primarily facultative bark dwellers but exclusive bark dwellers and species accidentally on bark were also present (Szinetár & Horváth 2006).

Conner et al. (1995) found only minimal use of nest-boxes by birds because their study area included four different mature forest types which were not intensively managed and which contained many dead trees and natural cavities. In contrast, all nest-boxes were used by birds for breeding in our intensive managed forest study area during both years of study; similar results have been found by other ecological studies in this area (e.g., Krištín & Žilinec 1997; Krištín et al. 2001).

Season and bark type.—Season significantly influenced abundance as well as composition of spider communities. The abundances of spiders in the nest-boxes peaked in autumn when spiders sought crevices for overwintering. Horváth at el. (2005) found that the abundance of spiders on pine, *Pinus nigra*, increased from summer to fall in urban sites, but decreased or stayed stable in forests. We also found both a clear taxonomic and functional turnover from season to

season in the spider communities. In spring, assemblages were dominated mainly by *Anyphaena accentuata* categorized as Other hunters. In summer, communities were dominated by Sheet web weavers from the family Linyphiidae and in autumn again by Other hunters but with spiders *Clubiona* spp., *A. accentuata*, and *Philodromus* spp. The community dynamic might be, at least partly, influenced by the interplay between phenology of spiders and IGP. The interior of the nest-boxes were relatively homogeneous as their sides were smooth. The nest-boxes were also relatively poor in alternative prey other than spiders. Thus, IGP among spiders could be intense (Finke & Denno 2006; Riekers et al. 2006). In our system, the Other hunters were larger than the Sheet web weavers during spring and autumn. In addition, *A. accentuata* and philodromids are winter-active and prey even at temperatures close to 0°C (Pekár et al. 2015; Petráková et al. 2016). The winter-active Other hunters can therefore substantially reduce the abundances of smaller spiders from autumn until spring (Gunnarsson 1985; Pekár 1999; Petráková et al. 2016). In addition, as *A. accentuata* was a top predator among spiders in our system, the predation on other spiders during winter can explain its dominance in the nest-boxes during spring. Indeed, *A. accentuata* excluded philodromids already in autumn if it reached high abundances. During summer, the IGP on the small linyphiids could be alleviated as the Other hunters were scarce and/or they were small.

Although we did not find a significant effect of bark type on abundances or taxonomic composition at the family level, we found increased dominance of Space web weavers in trees with rough bark in comparison to smooth bark. Small Space web weavers build their webs in bark crevices and therefore the attachment points and shelters provided by the rough bark may represent a limiting resource for them (Roberts 1996; Goméz et al. 2016). Their increased abundance in trees might then translate into higher abundances of Space web weavers within nest-boxes.

Predator effect.—While spiders also occupied nest-boxes that were used by birds breeding or roosting, the presence of avian predators decreased the abundance of spiders in nest-boxes by 67%. This decline in spider abundance can be explained by diets of the birds using these nest-boxes. Species *P. major* and *F. albicollis*—common occupiers of nest-boxes in our study—are among the most important predators of spiders (Gajdoš & Krištín 1997; Krištín 2002, Pagani-Núñez et al. 2011). Although the adult birds do not usually forage within nest-boxes, older nestlings sometimes do (Krištín, unpubl.). Birds also may prey in trees nearby active nest-boxes and spider assemblages in the nest-boxes likely represent a sub-set of nearby available species and individuals. Moreover, the effect of birds on spiders may include also non-consumptive factors such as their mere presence, movement within nest-boxes, and web destruction which may induce spider emigration (Chmiel et al. 2000; Werner & Peacor 2003; Goncalves-Souza et al. 2008; Mestre et al. 2014; Bucher et al. 2015).

We found no significant difference in the kinds of primary defense mechanisms of spiders in comparing protected and unprotected nest-boxes. The defense mechanisms of spiders observed in our study are most effective against visually oriented predators (Pekár 2014). The insides of nest-boxes are

very dark and certain defense mechanisms may not function well under such dark conditions. Alternatively, all mechanisms were equally effective against avian predators. Nevertheless, we found that the presence of predators affected the community composition with respect to spider guilds, which is known to influence the risk of being preyed upon by birds (Gunnarson 2007; Mestre et al. 2013; Gunnarson & Wiklander 2015). Gunnarson & Wiklander (2015) have found that Hunters are exposed to the higher predation risks than Web weavers. One explanation provided by those authors was that the webs can protect spiders from birds. In contrast, we found that the presence of birds reduced the proportions of Sheet web weavers and Sensing web weavers, while the proportion of Hunters increased. Similar pattern was observed by Mestre et al. (2013). The stronger effect on web weavers in our study can be due to destruction of webs by birds, which led to the spider emigration. The web weavers might also be outside their webs where they are clumsier than hunters and are, therefore, more prone to bird predation.

Intraguild predation.—We found a significant relationship between *A. accentuata* and philodromids, which was influenced by the bark type. The influence of bark type on this relationship indicates that the processes at the tree scale influenced the community composition at the nest-box scale. In the rough bark, there was a hump-shaped relationship between abundances of philodromids and *A. accentuata*. This means that both species positively responded to some factors at first (e.g., prey availability, suitable microhabitat conditions, vertebrate predator free space), but as the interference intensified *A. accentuata* excluded the philodromids. In the smooth bark, the abundances of philodromids basically only declined with increasing abundance of *A. accentuata*. The rough bark reduced the intensity of IGP because it can provide small crevices that are not accessible for large *A. accentuata* and so they can serve as the enemy-free shelters for the smaller philodromids (Korenko & Pekár 2010). The exclusion of philodromids could be due to consumptive effect as predation evinced by *Anyphaena* on philodromids can be severe (Korenko & Pekár 2010; Petráková et al. 2016). The exclusion can be also due emigration caused by trait-mediated effect or direct non-consumptive interference (Schmidt & Rypstra 2010; Mestre et al. 2014; Schmidt et al. 2014).

In conclusion, our study provides a better understanding of the processes affecting the distribution of spider assemblages in tree cavities. The community of spiders in nest-boxes was affected by season, bark type, predation by birds, IGP among spiders and interaction among the abiotic and biotic factors depending on spider functional traits, namely guild association and size. Our results from the exclosure experiment support strong negative effects of birds especially on web-building spiders. Finally, our study suggests that use of nest-boxes is an effective method for evaluation of arboreal spider assemblages. Nest-boxes provide refuge for spiders and serve as useful alternatives to tree hollows and tree bark.

ACKNOWLEDGMENTS

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Appendix 1.—Spider guilds, defense mechanism and number of individuals sampled in nestboxes during all seasons with emphasis on bark type in 2012, and in protected (fenced) and unprotected nest-boxes during exclosure experiment in 2013 in two mixed forests.

Family	Season			Bark type		Experiment			
Species	Spring	Summer	Autumn	Rough	Smooth	Unprotected	Protected	Guilds	Defense mechanism
Dysderidae									
Harpactea hombergi (Scopoli, 1763)	0	0	0	0	0	7	11	specialist	batesian mimicry
Segestridae									
Segestria senoculata (Linnaeus, 1758)	0	7	9	3	13	3	3	sensing web	crypsis
Theridiidae									
Cryptachaea riparia (Blackwall, 1834)	0	1	0	0	1	0	0	space web	anachoresis
Platnickina tincta (Walckenaer, 1802)	2	1	43	23	23	0	3	space web	crypsis
Steatoda bipunctata (Linnaeus, 1758)	0	0	0	0	0	1	0	space web	anachoresis
Theridion mystaceum L. Koch, 1870	0	0	0	0	0	3	3	space web	crypsis
Theridion varians Hahn, 1833	0	1	0	1	0	0	0	space web	crypsis
Theridion spp.	1	3	10	10	4	5	5	space web	crypsis
Linyphiidae									
Drapetisca socialis (Sundevall, 1833)	0	5	0	4	1	1	2	sheet web	crypsis
Leptyphantes leprosus (Ohlert, 1865)	0	1	6	0	7	2	3	sheet web	anachoresis
Moebelia penicillata (Westring, 1851)	0	2	0	2	0	0	0	sheet web	not available
Leptyphantes spp.	4	16	5	13	12	5	4	sheet web	anachoresis
Araneidae									
Araneus diadematus Clerck, 1757	0	0	2	0	2	0	0	orb web	crypsis
Nuctenea umbratica (Clerck, 1757)	0	1	0	1	0	0	1	orb web	anachoresis
Agelenidae									
Agelena labyrinthica (Clerck, 1757)	0	0	1	0	1	0	0	sheet web	anachoresis
Tegenaria silvestris L. Koch, 1872	0	0	0	0	0	1	1	sheet web	crypsis
Tegenaria ferruginea (Panzer, 1804)	2	0	0	2	0	0	5	sheet web	crypsis
Amaurobiidae									
Amaurobius fenestralis (Ström, 1768)	2	2	15	7	12	4	14	sheet web	anachoresis
Anyphaenidae									
Anyphaena accentuata (Walckenaer, 1802)	91	3	69	87	76	10	5	other hunters	not available
Clubionidae									
Clubiona comta C.L.Koch, 1839	0	0	0	0	0	1	1	other hunters	anachoresis
Clubiona corticalis (Walckenaer, 1802)	0	0	2	0	2	1	1	other hunters	anachoresis
Clubiona marmorata L. Koch, 1866	0	0	1	1	0	1	0	other hunters	anachoresis
Clubiona pallidula (Clerck, 1757)	0	0	0	0	0	1	6	other hunters	anachoresis
Clubiona spp.	10	2	204	103	113	0	2	other hunters	
Gnaphosidae									
Arboricaria subopaca Westring, 1861	0	0	0	0	0	1	0	ground hunters	crypsis
Zelotes apricorum (L. Koch, 1876)	0	0	0	0	0	1	0	ground hunters	anachoresis
Haplodrassus sp.	0	0	0	0	0	1	0	ground hunters	not available
Scotophaeus sp.	0	1	3	2	2	2	2	ground hunters	anachoresis
Philodromidae									
Philodromus aureolus group	2	1	27	24	6	0	0	other hunters	crypsis
Philodromus margaritatus (Clerck, 1757)	0	0	18	4	14	0	0	other hunters	crypsis
Thomisidae									
Diaea dorsata (Fabricius, 1775)	0	0	2	2	0	0	0	ambush hunters	crypsis
Ozyptilla sp.	0	0	0	0	0	1	1	ambush hunters	not available
Xysticus sp.	0	5	1	2	4	2	2	ambush hunters	crypsis
Salticidae									
Ballus chalybeius (Walckenaer, 1802)	0	1	0	0	1	0	3	other hunters	batesian mimicry
Heliophanus dubius C. L. Koch, 1835	0	0	0	0	0	1	0	other hunters	batesian mimicry
Marpissa muscosa (Clerck, 1757)	2	0	0	1	1	1	1	other hunters	crypsis
Pseudeophrys erratica (Walckenaer, 1826)	0	6	0	0	6	1	5	other hunters	not available
Salticus scenicus (Clerck, 1757)	0	0	0	0	0	1	0	other hunters	crypsis
Salticus zebraneus (C. L. Koch, 1837)	0	0	0	0	0	1	0	other hunters	crypsis
Total	**116**	**59**	**418**	**292**	**301**	**44**	**92**		

Microhabitat use in the amblypygid *Paraphrynus laevifrons*

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Abstract.   Amblypygids (Order: Amblypygi) can be found across different habitat types, each with very different microhabitat structure, including rainforests, deserts, and caves in the tropics and subtropics. Most prior studies on amblypygid microhabitat use have focused on characteristics of trees and their relationship with amblypygid abundance, though many species regularly occupy refuges away from trees. Here we explore microhabitat use in the amblypygid *Paraphrynus laevifrons* Pocock, 1894 through mark-recapture surveys conducted along creeks and trails in a secondary forest in southeastern Costa Rica. We identified (1) microhabitat characteristics associated with abundance of *P. laevifrons* and (2) resighting ratio—the likelihood of finding individual *P. laevifrons* over multiple nights, potentially in association with a particular area (a putative territory). We measured four microhabitat characteristics: (i) number of visible refuges, (ii) surface area of vertical substrate, (iii) estimated plant cover of substrate, and (iv) presence/absence of an overhang. We found that the number of *P. laevifrons* sighted did not differ across wet and dry seasons, but *P. laevifrons* were sighted in greater numbers in creeks than trails. The abundance of *P. laevifrons* was positively affected by the presence of overhangs, there was no effect of plant cover, and the positive effect of number of refuges was stronger in trails, where overhangs were less common, than in creeks. Our results support earlier studies showing that amblypygids can be found more abundantly in areas with greater available refuges and potential shelter, suggesting that predation may be a strong source of selection on amblypygid microhabitat use.

Keywords: Microhabitat preference, natural history, whip spider, movement patterns

Arachnids occupy a tremendous range of habitats, and habitat and microhabitat use has been studied in diverse arachnid taxa. While broad-scale studies of spiders have demonstrated how species presence and density differ across habitat types (Jordan et al. 1994; Aiken & Coyle 2000; Bonte & Maelfait 2001; Pearce et al. 2004; Lapinski & Tschapka 2013), finer-scale studies show how factors such as prey abundance (Harwood et al. 2003; Johnson et al. 2011), anthropogenic building materials (Fischer et al. 2005), proximity to water (DeVito et al. 2004; Lapinski & Tschapka 2014), temperature, and humidity (Yáñez & Floater 2000) affect microhabitat preference within these areas. Further, predictable changes in the environment may lead to seasonal patterns of (micro)habitat use within species (Arango et al. 2000). Together, such studies provide important information regarding the ecology and natural history of spiders, yet much of this basic information is lacking in other arachnid groups.

Like their spider relatives, amblypygids (Order: Amblypygi) are found across a wide range of habitats, including rainforests, deserts, and caves in the tropics and subtropics (reviewed in Weygoldt 2000). Their dorsoventrally flattened bodies allow them to occupy narrow crevices in and around rocks, fallen logs, and at the base of trees (Weygoldt 1977, 2000). Their antenniform front legs are covered in mechano- and chemosensory hairs used in olfaction, touch, and contact chemoreception (Weygoldt 2000; Foelix & Hebets 2001; reviewed in Santer & Hebets 2011), and have been demonstrated to facilitate the learning and discrimination of tactile cues associated with available refuges (Santer & Hebets 2009).

Amblypygids are presumed to be territorial (Hebets 2002; Porto & Peixoto 2013; Chapin & Hill-Lindsay 2015). Previous studies in the amblypygid *Phrynus pseudoparvulus* Armas & Víquez, 2002, for example, have shown that individuals not only occupy home refuges for extended periods of time (weeks–months) but they may travel far distances away from

this home refuge, only to return weeks later (Hebets 2002). Further field studies in the amblypygid *Heterophrynus longicornis* Butler, 1873 suggest that individuals may select and defend territories based on the presence of burrows; released individuals were found and remained at sites with burrows present, and when large individuals were removed from a putative territory, smaller individuals took their place (Porto & Peixoto 2013). In addition to field data on movement patterns, ritualized agonistic interactions are commonly observed between conspecific amblypygids; and these ritualized displays during intrasexual interactions have been hypothesized to play a role in defense of a home territory (*Phrynus marginemaculatus* C.L. Koch, 1840, Fowler-Finn & Hebets 2006; *Heterophrynus longicornis*, Porto & Peixoto 2013; *Phrynus longipes* Pocock, 1894, Chapin & Hill-Lindsay 2015; reviewed in Santer & Hebets 2011 and Chapin & Hebets 2016). Given the suggestion of territoriality and the observations of agonistic interactions between conspecifics in some species, it seems likely that there is competition for resources in their environment.

Previous studies on amblypygid microhabitat use have found that amblypygid species in New World tropical and subtropical rainforests prefer trees that are large and/or have an abundance of burrows or refuges (Hebets 2002; Dias and Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013; Chapin 2014; Curtis & Bloch 2014). Hebets (2002) found a positive correlation between the number of *P. pseudoparvulus* sighted and the surface area of trees; individuals were more likely to be resighted on trees with greater surface area, moss cover, and buttressing. Carvalho et al. (2012) found *H. longicornis* more abundantly in forest fragments with a greater number of trees and on trees with termite nests; however, individuals were found more frequently on relatively small trees (DBH between 10–50 cm). Together, these studies suggest that multiple microhabitat characteristics may affect

habitat use in different ways for different amblypygid species. But while most prior studies have focused on the characteristics of trees within primary lowland tropical forests and their relationship with amblypygid abundance, many amblypygid species regularly occupy refuges away from trees (reviewed in Weygoldt 2000) and/or occupy multiple microhabitat types. In the present study, we performed mark-recapture surveys across two microhabitat types (creeks and trails) within a secondary lowland tropical rainforest in southeastern Costa Rica for a widespread amblypygid species, *Paraphrynus laevifrons* Pocock, 1894.

Paraphrynus laevifrons is found across Costa Rica and into Panama (Mullinex 1975; Viquez pers. comm.). Individuals can be found on vertical surfaces, including the base of trees and the sides of creeks, in tropical wet forests (Corey pers. obs.). Through our surveys, we aimed to identify (1) microhabitat characteristics associated with abundance of *P. laevifrons*, and (2) resighting ratio – i.e., the likelihood of finding individual *P. laevifrons* over multiple survey nights, potentially in association with a particular area (a putative/potential territory).

METHODS

Study area.—This study took place at Las Cruces Biological Station in Coto Brus county, Costa Rica. The areas surrounding the biological station are predominantly made up of selectively-logged primary, tropical wet forest and secondary forest. To examine microhabitat use within and across creeks and trails, we established three non-overlapping survey plots, each containing a paired creek and trail transect. In total then, we surveyed three creek transects and three trail transects (3 pairs of transects; one pair within a "survey plot"). Two survey plots had transects that were perpendicular to each other, and one survey plot had transects that were parallel.

Creek and trail transects were 50 m x 3 m, which in practice included only one side of a given creek or trail due to their width. We included the side with more measurable vertical substrate, and therefore more potential microhabitat for *P. laevifrons*, following our preliminary observations that *P. laevifrons* are found on vertical surfaces. To obtain more accurate measurements of microhabitat characteristics and locations of individual *P. laevifrons*, we divided each individual creek and trail transect into approximately 5 m-long "survey grids," with a total of ten survey grids nested within each transect. Creek and trail transects within a survey plot were close (as close as 1 m at some points), but did not directly overlap, leaving the possibility that individuals could move between transects. During the course of our surveys, and during additional fieldwork at the station, *P. laevifrons* was the only amblypygid species identified.

Microhabitat characteristics.—In creek and trail transects, we measured the following four microhabitat characteristics: (i) number of visible refuges, (ii) surface area of vertical substrate (i.e., area of exposed creek/trailside that individuals could climb on), (iii) estimated plant cover of substrate, and (iv) presence/absence of an overhang.

We define a (i) visible amblypygid refuge as an area large enough for at least a small juvenile *P. laevifrons* to enter and remain covered (e.g., an abandoned animal burrow or small crevice) with only one visible entrance in the side of the

vertical substrate. To estimate (ii) surface area of the vertical substrate (i.e., the amount of exposed and/or plant-covered soil on creek/trailsides along transects), we measured the height of transects at two points within each survey grid – approximately 1 meter from the start and from the end of the survey grid – and averaged the two heights. We then multiplied the average height by the width of each survey grid (approximately 5 meters) to obtain vertical surface area. We only found *P. laevifrons* on vertical surfaces in preliminary surveys, and therefore only sampled these vertical surfaces in this study. Similar to Hebets (2002), we estimated (iii) plant cover within survey grids as the amount of living and dead plant material (leaves and stems) covering the vertical substrate, in the following ranges: 0–25%, 25–50%, 50–75%, and 75–100% cover. We considered (iv) an overhang to be present in an individual survey grid if the majority of vertical substrate (over 50% of the survey grid width) had above it a horizontal surface that protruded beyond the base of the creek/trailside, usually comprised of compacted soil and roots.

Mark-recapture surveys.—We performed mark-recapture surveys between 11–21 August 2015 (wet season), and 6–10 January 2016 (dry season). Surveys took place at night, between 2000 and 0500 hours (wet season) and 1925 and 2400 hours (dry season). We surveyed the same six transects (creek = 3, trail = 3) across wet and dry seasons. Across seasons, each creek and trail transect was surveyed three times over three different nights. During the wet season, we surveyed transects in similar microhabitats in a given night – i.e., all 3 creek transects or all 3 trail transects – for a total of three survey nights per transect (and per microhabitat type). During the dry season, we surveyed transects within a shared survey plot (creek + trail) on any given night and 2–3 of our survey plots were surveyed per night. This difference in surveying procedure was due to greater time constraints on fieldwork in the wet season and a reassessment of best survey protocols following the wet season surveys. Despite subtle differences in methodology, we have a total of 3 survey nights for each of our 3 creek and 3 trail transects for both wet and dry seasons.

We surveyed for *P. laevifrons* using a headlamp with white light, and examined each individual survey grid from beginning to end at least twice per survey, moving from the beginning to the end of the transect. The total time spent conducting nightly surveys varied depending on the number of *P. laevifrons* sighted and captured. Whenever we found an individual, we recorded the survey grid that it was located in as well as a general description of its location (e.g., under an overhang, adjacent to a small crevice, on an exposed root). We opportunistically hand-captured as many individuals as possible and recorded their cephalothorax width (in mm, using Control Company Traceable® 150 mm digital calipers), age class (juvenile or adult), and labeled them with an identification code using Sharpie non-toxic oil-paint markers. Age class was identified using both individual size and coloration. Juveniles of *P. laevifrons* have pronounced red pedipalps and a yellow perimeter around the dorsal side of their cephalothorax, while adults typically have pedipalps that are gray-brown, similar to the rest of their bodies, and a more subdued color to the perimeter of their cephalothorax (Corey pers. obs). Previous studies show that related amblypygids reach sexual maturity when they have achieved a cephalotho-

Table 1.—Summary of mark-recapture surveys for juvenile and adult *Paraphrynus laevifrons* along creek and trail transects during wet (August) and dry (January) seasons. See Methods for details on calculating ratio of individuals resighted (here expressed as a percentage), estimated population size and estimated population density (individuals/m^2), ± standard deviation. Where standard deviations are not shown, we could only calculate one estimate pool as no individuals were resighted in that sample (see Methods). Individuals were more often resighted in trails than creeks, but there were no differences across age classes and seasons (see Table 3).

Age	Season	Micro-habitat	Number Sighted	Number Captured	Percent Resighted	Estimated Pop. Size	Estimated Pop. Dens.
Juvenile	Wet	Creek	56	23	25.0%	170.0 ± 116.0	0.61 ± 0.42
		Trail	27	9	100.0%	17.3 ± 1.9	0.11 ± 0.02
		Total	83	32	55.0%	91.7 ± 111.0	0.21 ± 0.02
	Dry	Creek	55	25	35.0%	142.5 ± 111.0	0.51 ± 0.40
		Trail	12	5	20.0%	35.0	0.21
		Total	67	30	32.0%	249.9 ± 219.4	0.57 ± 0.06
Adult	Wet	Creek	44	22	27.3%	97.0 ± 18.4	0.35 ± 0.07
		Trail	12	7	80.0%	3.8 ± 1.8	0.02 ± 0.01
		Total	56	29	43.8%	91.8 ± 11.6	0.21 ± 0.03
	Dry	Creek	48	20	29.4%	76.5 ± 61.5	0.28 ± 0.29
		Trail	11	3	33.3%	7.0	0.04
		Total	59	23	30.0%	73.9 ± 26.3	0.17 ± 0.06

rax width greater than 10 mm (*Phrynus marginemaculatus*, Weygoldt 2002); therefore, we identified all captured individuals with a cephalothorax width greater than 10 mm to be adults, and those with smaller cephalothoraxes to be juveniles.

Resighting ratio was calculated for individual age classes and seasons by adding the number of previously marked individuals sighted on the second and third (final) survey nights across transects, and dividing by the number of individuals marked on the first and second survey nights. The estimated population size across transects was calculated using the Multiple Lincoln-Petersen estimator (following Grimm et al. 2014). The Multiple Lincoln-Petersen estimator pools multiple survey samples into $k - 1$ estimates (where k is the number of survey samples) from which the average and variance are then calculated. In our study, $k = 3$ samples per microhabitat per season, so we therefore have two estimates that we use in calculating the average population size and its variance (here expressed as standard deviation). In the Lincoln-Petersen model, $N = (n_1 * n_2) / m_2$, where N is the estimated population size, n_1 is the number of individuals marked in an initial survey (the number of captured *P. laevifrons* on the initial survey night in a transect), n_2 is the size of the subsequent survey sample (the total number of sighted *P. laevifrons* in the subsequent survey night in a transect), and m_2 is the recapture ratio (the number of previously marked individuals resighted in a subsequent survey night in a transect). For the Multiple Lincoln-Petersen estimator, we formed two different pools, where (1) survey nights 1 and 2 are combined as the initial survey and survey night 3 is the subsequent survey, and (2) survey night 1 is the initial survey and survey nights 2 and 3 are combined as the subsequent survey. Population densities of *P. laevifrons* (individuals/m^2) across age classes (juvenile/adult), microhabitats, and seasons were estimated by dividing population sizes using the Multiple Lincoln-Peterson estimator by the surface area across transects (Creek = 277.5 m^2, Trail = 163.5 m^2, Total = 441 m^2).

The Lincoln-Petersen model assumes that (i) the population is closed to recruitment, death, immigration, and emigration, (ii) capture probability is equal among animals in each sample, and (iii) all marked animals are found in subsequent surveys

(reviewed in Pine et al. 2003). This estimator allows for the pooling of mark-recapture data across greater than two survey samples, and is therefore more robust to violations of the assumption that capture probability is equal among animals in each sample (assumption ii above), by increasing the capture probabilities and decreasing the range of capture probabilities (Grimm et al. 2014). Nevertheless, given that these are strict assumptions, and likely to be violated, we present these data as rough, preliminary estimates.

Statistical analyses.—Differences in abundance across seasons (wet vs. dry), and differences in the number of refuges, density of refuges, and plant cover across microhabitat types, were compared using a Mann-Whitney U Test. We used a chi-squared test to compare differences in the frequency of overhang presence across microhabitat types.

We analyzed the effects of microhabitat characteristics on abundance of *P. laevifrons* using a negative binomial-distributed, generalized linear mixed model. The response variable in the model was the number of individual *P. laevifrons* (both adults and juveniles) sighted per survey grid on a given survey night. We use this measure of abundance, individuals sighted *per survey grid*, because this is the spatial scale at which we measured the microhabitat characteristics. The model contained fixed effects terms for the number of refuges, vertical surface area, estimated plant cover (as a continuous variable, using the midpoint of each estimated range), microhabitat type (creek/trail), and overhang (present/absent). We specified an interaction term between microhabitat type and overhang presence, and microhabitat type and number of refuges following preliminary analyses. Given that vertical surface area differed across microhabitat types, we use surface area as a means of controlling for differences in sampling effort across microhabitat types. That is to say, we can examine how much variation in the abundance of *P. laevifrons* can be attributed to variation in microhabitat characteristics after controlling for the variation in response to surface area. The model also contained a random effects term for survey night nested within transect, specifying randomly varying intercepts, to control for repeated sampling of the same transects across survey nights (Table 2).

Table 2.—Effects of microhabitat characteristics on abundance of *Paraphrynus laevifrons* (negative Binomial-distributed, generalized linear mixed model). Estimates are the number of individuals found per 5 meter long survey grid along study transects. Values in **bold** are significant at $p = 0.05$.

Fixed Effects	Estimate	Std. Error	z value	$P(>|z|)$
Overhang (Present)	1.138	0.245	4.64	**<<0.001**
Microhabitat (Trail)	−0.847	0.351	−2.41	**0.016**
Number of Refuges	0.023	0.012	1.91	0.056
Vertical Surface Area	0.049	0.018	2.65	**0.008**
Plant Cover	−0.002	0.003	−0.54	0.593
Microhabitat * Overhang	−0.349	0.472	−0.74	0.460
Microhabitat * Number of Refuges	0.038	0.018	2.11	**0.035**
Random Effects	Variance	Std. Dev.		
Survey Night : Transect	<<0.001	<<0.001		
Transect	0.008	0.089		

We used a binomial-distributed generalized linear mixed model with a response variable of resighting a marked individual (yes/no) to examine differences in the ratio of resighted individuals. This model contained fixed effects terms for the age class of individuals (juvenile/adult), season (wet/dry), and microhabitat type (creek/trail). We also included a random effects term for transect surveyed, specifying randomly varying intercepts (Table 3). All statistical analyses were performed using R version 3.3.2.

RESULTS

The number of *P. laevifrons* sighted did not significantly differ across seasons (Mann Whitney U test, W = 16,449, P = 0.770; Fig. 1), but *P. laevifrons* were sighted in greater numbers in creeks than trails (Tables 1, 2). During the wet season, 139 *P. laevifrons* (57 adults and 82 juveniles) were sighted, of which 61 were captured and labeled. During the dry season, 126 *P. laevifrons* (59 adults and 67 juveniles) were sighted, of which 51 were captured and labeled (Table 1).

The number of visible refuges was greater in creeks than trails (Mann Whitney U Test, W = 657, P = 0.002). However, refuge density did not differ between creeks and trails (Mann Whitney U Test, W = 455, P = 0.5996), suggesting that this pattern is driven by differences in vertical surface area, which was greater in creeks. Plant cover did not differ between creeks

Table 3.—Differences in resighting ratio as affected by age class (juvenile/adult), microhabitat type (creek/trail), and season (wet/dry) (Binomial-distributed, generalized linear mixed model). Estimates are probability of resighting a previously marked individual *Paraphrynus laevifrons*. Values in **bold** are significant at $p = 0.05$.

Fixed Effects	Estimate	Std. Error	z value	$P(>|z|)$
Microhabitat (Trail)	1.417	0.553	2.58	**0.010**
Age (Juvenile)	0.186	0.491	0.38	0.705
Season (Wet)	0.592	0.491	1.21	0.228
Random Effects	Variance	Std. Dev.		
Transect	0	0		
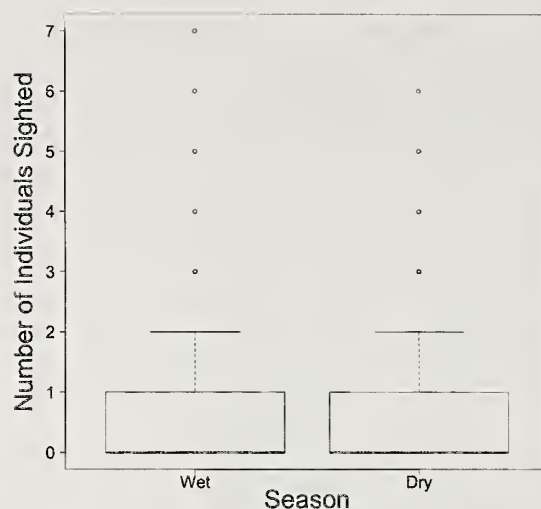
Figure 1.—Box-and-whisker plot of individual *Paraphrynus laevifrons* sighted in a given 5 meter-long survey grid per transect survey. The average number of *P. laevifrons* sighted did not differ between the wet season (August) and the dry season (January). Dots represent survey grid observations in which the number of individuals sighted was greater than the 75th percentile for a given season.

and trails (Mann Whitney U Test, W = 384.5, P = 0.3139) and overhangs were present more frequently in creeks than trails (Chi-squared test, $\chi^2 = 12.129$, P = 0.0005).

The differences in surface area between microhabitat types explained a large amount of the variation in the abundance of *P. laevifrons* (Table 2). After controlling for this difference, we found that the total abundance of *P. laevifrons* was positively affected by the presence of an overhang (Fig. 2, Table 2), while there was no effect of plant cover on abundance (Table 2). The number of refuges alone was not found to be a significant predictor of *P. laevifrons* abundance in our analyses (P = 0.056), though we did find a significant interaction between microhabitat type and the number of refuges (Table 2). Specifically, we found that the positive effect of refuge number on abundance of *P. laevifrons* was stronger in trails than in creeks.

Resighting ratio was lower in creeks than trails, but did not differ across age classes or seasons (Tables 1, 3). All individuals resighted in the same season were found in the same or a neighboring survey grid. We identified two adult *P. laevifrons* that were observed in both seasons – one of these individuals was found in the same transect, and the other was found in the creek transect neighboring the trail transect in which it was originally sighted.

Our preliminary findings on population density, as estimated using the Multiple Lincoln-Petersen estimator, suggest that density may differ across age classes and microhabitat types (Table 1), though these patterns may be similar across wet and dry seasons. Specifically, juvenile *P. laevifrons* may be found in greater population densities than adults, and both age classes are found in greater densities in creeks than trails.

Given that we opportunistically collected individuals and measured their size, we were able to obtain additional information regarding the phenology of *P. laevifrons*. We found one adult carrying brood on its opisthosoma during our January surveys and we captured 5 juveniles with cephalo-
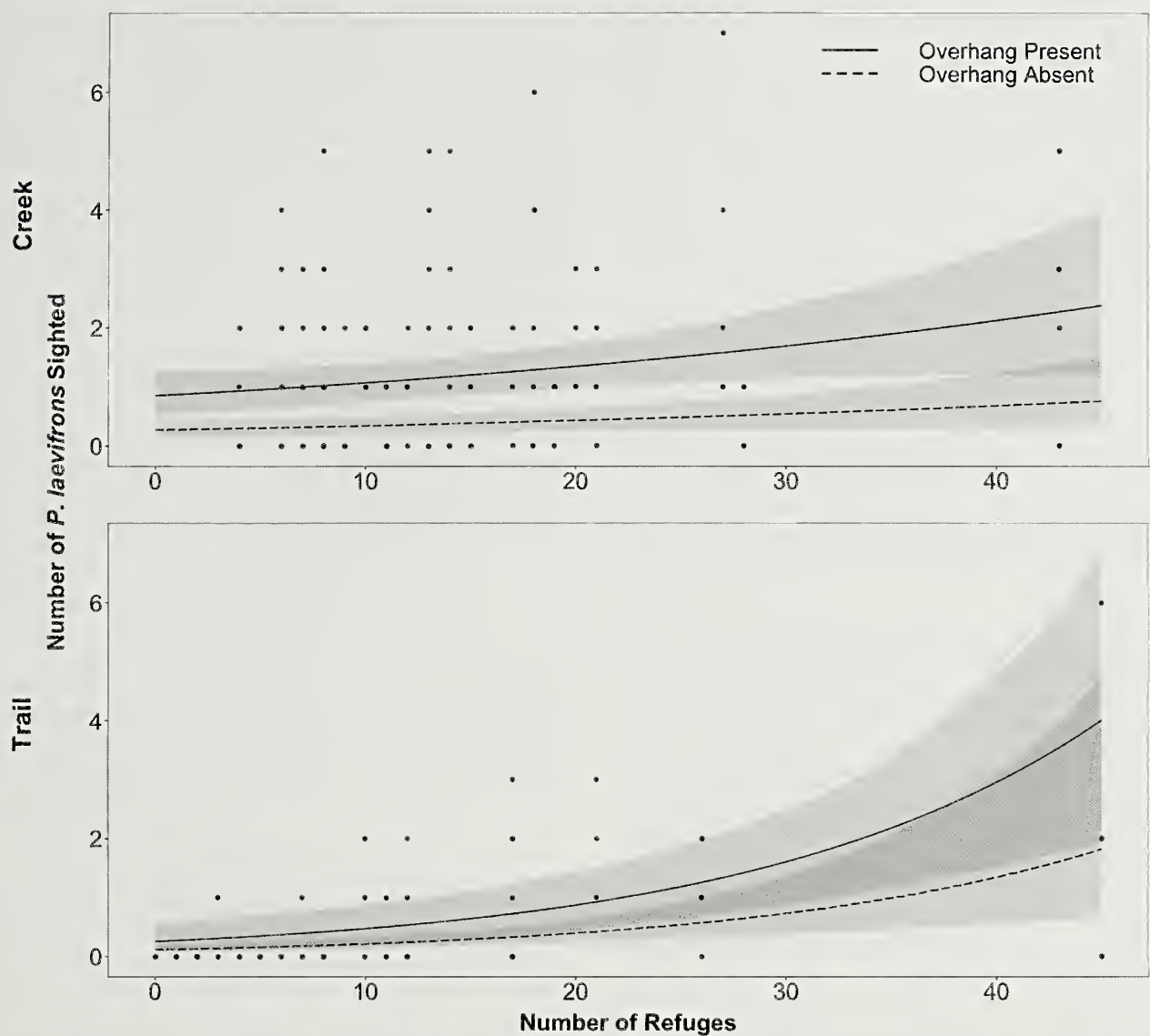
Figure 2.—The number of *P. laevifrons* sighted during surveys within a five meter-long survey grid was positively affected by the number of visible refuges and the presence of an overhang over the majority of the survey grid. There was an interaction between the number of refuges and microhabitat type, but no interaction between overhang presence and microhabitat type. Each dot represents an individual survey night observation for a grid containing the number of refuges indicated by the x – axis. Each individual survey grid was examined three times each in the wet and dry seasons, all on separate nights. Shaded areas represent 95% confidence intervals (n = 1000 bootstrap simulations per prediction line).

thorax widths between 3 and 4 mm in January (minimum = 3.41 mm), while no individuals this small were found during our surveys in August (minimum = 4.76 mm) (Fig. 3). These are likely very young individuals, who may have relatively recently left their mother's opisthosoma (Weygoldt 2000). We also found multiple small juveniles in the same survey grid as a single, large adult several times during January surveys. In subsequent collecting trips (3–11 June 2016), two females with egg sacs were collected, and three individuals formed egg sacs while in captivity between June and mid-August.

In addition, we colleeted three individuals during the wet season that were carrying the pupae of parasitic flies (family: Chloropidae) on the dorsal side of their opisthosoma (including one individual with its dorsal prosoma covered in pupae as well). We housed individuals until the adults eelosed,

and identified these flies as *Pseudogaurax sp.* based on previous descriptions by Viquez & DeArmas (2009).

DISCUSSION

Individuals of the amblypygid *Paraphrynus laevifrons* were commonly found along creeks and trailsides at Las Cruces Biological Station in a tropical rainforest in Costa Rica. Our surveys indicated that individuals were in higher abundance along creek transects as compared to trail transects (Table 2), and this difference was consistent across wet and dry seasons. Our observed differences in abundance are likely driven, in part, by differences in microhabitat characteristics – specifically surface area, overhangs, and refuges. Differences in surface area between creeks and trails explained a large
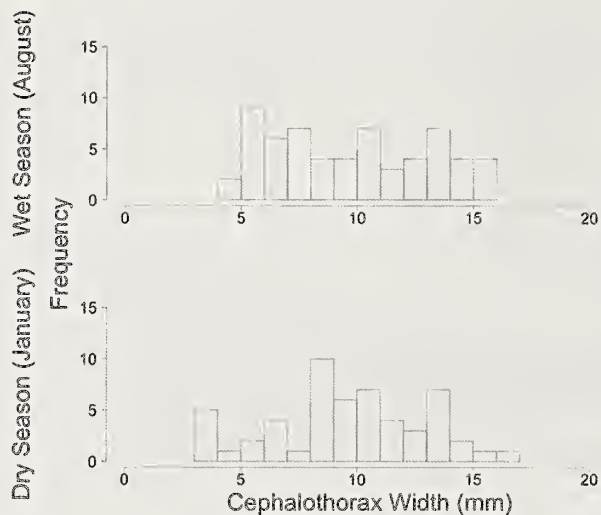
Figure 3.—Cephalothorax widths (mm) of *Paraphrynus laevifrons* captured, marked, and released during mark-recapture surveys in the wet season (August) and dry season (January). The range of sizes was greater in the dry season.

amount of the variation in the abundance of *P. laevifrons* (Table 2). After controlling for this confounding variable, we found that the presence of overhangs was the best predictor of amblypygid abundance, with a greater frequency of overhangs associated with more amblypygids. While the number of refuges alone was not predictive of amblypygid abundance, we found a significant interaction term between microhabitat type and number of refuges (Table 2). Specifically, the positive effect of refuges on abundance was greater in trails than in creeks. While the total number of refuges was greater in creeks, refuge density (the number of refuges divided by vertical surface area) did not differ across microhabitat types, suggesting that the greater difference of vertical surface area in creeks may be driving this difference. Given that overhangs are more common in creeks than trails (60% of creek survey grids had overhangs, compared to only 13.3% of trail survey grids), but refuges are similarly dense in creeks and trails, refuges may be a more important source of shelter for *P. laevifrons* along trailsides than in creeks. That is, in the absence of potential cover provided by overhangs, refuges may play a stronger role in microhabitat selection for *P. laevifrons*. This would be consistent with previous studies that have demonstrated how the abundance of refuges plays an important role in microhabitat use in amblypygids (Dias & Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013).

Our population density calculations suggest higher densities of juveniles than adults, with both age classes found in greater densities in creeks than trails (Table 1). The ratios of marked, resighted individuals were less in creeks than in trails, but did not differ across age classes or seasons (Tables 1, 3). The greater resighting ratio along trails may suggest greater site fidelity in this microhabitat (which has been demonstrated in other amblypygid species, see Hebets 2002; Hebets et al. 2014). Given that population densities of *P. laevifrons* appear to be lower along trails (see below), individuals may be able to maintain territories longer due to a reduced encounter rate with conspecifics. This hypothesis requires testing. Additionally, it is important to note that this study reflects a very small

time window of observation; further longer-term observations are required to generate a complete picture of microhabitat use and movement patterns in *P. laevifrons*.

Previous microhabitat preference studies on amblypygids in the genus *Heterophrynus* found that individuals prefer trees with buttressing and many crevices (Dias & Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013; Chapin 2014). Our surveys focused solely on creeks and trails, as preliminary forest transect surveys found only two individuals across 71 trees (unpublished data). The secondary forest has been selectively logged at our field site, and presumably the largest trees (containing potentially high-quality microhabitat) were removed. Selective logging has been demonstrated to decrease population density in the amblypygid *Phrynus longipes* (Bloch & Weiss 2002). Nonetheless, similar to other studies, our results suggest that diurnal refuges and microhabitat characteristics that offer shelter are the most important predictors of amblypygid abundance. At our field site, these characteristics are most prevalent along creeks. Future studies should examine microhabitat use in *P. laevifrons* in undisturbed primary forests, as our observations of microhabitat use may reflect the species' strategy for coping with anthropogenic disturbance rather than preferred microhabitat use.

Our present study focused only on the structural microhabitat characteristics that affect individual abundance in *P. laevifrons*. However, the physiology of *P. laevifrons* may interact with microhabitat characteristics to affect individual abundance and/or density. For example, Lapinski & Tschapka (2014) found that desiccation tolerance reflects the microhabitat association patterns of an assemblage of tropical wandering spiders – specifically, that semi-aquatic and forest-ground-dwelling spiders had high water loss rates and desiccation susceptibility, which is consistent with their humid microclimate. Such a limitation may well exist in *P. laevifrons*, as individuals are very sensitive to humidity while in captivity. While we did not measure how humidity differs across creeks and trails, or in areas within these microhabitats that vary in the number of overhangs or refuges, we suspect that humidity is higher in creeks than trails. Future work may confirm if desiccation tolerance and relative humidity may contribute to the abundance patterns we have observed.

Differences in abundance across microhabitat types are also likely influenced by factors such as prey and predator abundance (see Harwood et al. 2003). We have little beyond anecdotal evidence of the natural prey and predators of amblypygids, so collecting this information would be broadly applicable to amblypygid ecology (reviewed in Chapin & Hebets 2016). During our nightly surveys, we observed two *P. laevifrons* foraging – one capturing an ant (unknown species) on a trail transect and one consuming a cricket (unknown family) on a creek transect. We also observed numerous individuals consuming unidentifiable, macerated prey items. No instances of predation on *P. laevifrons* were observed over the course of this study.

Our opportunistic observations of individuals of different size throughout the seasons provide us some insight into potential reproductive seasons for *P. laevifrons*. First, during the dry season, we found multiple small juveniles in close proximity with a single, large adult. Previous studies on captive amblypygids suggest that there may be prolonged

mother-offspring-sibling associations (*Phrynus marginemaculatus* and *Damon diadema* Simon, 1876, Rayor & Taylor 2006) and offspring may be capable of kin recognition (*D. diadema,* Walsh & Rayor 2008). We propose that these small individuals were offspring of the adult female. Additionally, in subsequent collecting trips (3–11 June 2016), two females with egg sacs were collected, and three individuals formed egg sacs while in captivity between June and mid-August. While it is unknown how long eggs of *P. laevifrons* take to hatch, in other species, offspring emerge three to three and a half months after eggs are laid (Weygoldt 2000). Taken together, our observations suggest that *P. laevifrons* has a similar breeding season to that suggested for *Phrynus pseudoparvulus* – namely a primary breeding season from October to January, with some individuals breeding throughout the year (Hebets 2002).

In summary, this study adds to a slowly growing body of literature focused on amblypygid behavioral ecology (Chapin & Hebets 2016). Our results are consistent with earlier studies showing that amblypygids can be found more abundantly in areas with greater available refuges and potential shelter (Dias & Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013), suggesting that predation may be a strong source of selection on amblypygid behavior. Unfortunately, little is known about the nature or abundance of amblypygid predators. Future fieldwork exploring natural predator-prey interactions—focusing on amblypygids as both predators and prey—as well as territorial behavior will help place our microhabitat use data in the broader context of amblypygid natural history.

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SHORT COMMUNICATION

Field observations of simultaneous double mating in the wolf spider
Rabidosa punctulata (Araneae: Lycosidae)

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Abstract. Males of many species of spider engage in alternative mating tactics that do not involve pre-mating courtship. Here I report field observations of a novel opportunistic mating tactic of the wolf spider *Rabidosa punctulata* (Hentz, 1844): simultaneous double mating, whereby males that encounter copulating pairs also mount and achieve inseminations concurrently with the first male. On three separate occasions, female *R. punctulata* were observed mating with two males simultaneously. Males that mate with already copulating females likely receive multiple fitness benefits. It may allow courtship parasitism of other males while also reducing male agonistic interactions, eliminate the need to court or subdue the female, and reduce pre-mating cannibalism risk. If such behavior is common, it may limit sexual selection acting on male courtship displays by reducing the effectiveness of pre-mating female choice while also increasing sperm competition.

Keywords: Polygynandry, lycosid, threesome, satellite male, sperm competition

Males of many species of spider engage in alternative or opportunistic reproductive tactics to maximize fitness under different conditions (reviewed in Robinson & Robinson 1980; Christenson 1984). Males may engage in sneak copulations (Elgar & Fahey 1996; Schneider et al. 2005), mate with recently molted (Jackson 1986) or feeding females (Prenter et al. 1994; Elgar & Fahey 1996; Fromhage & Schneider 2005) or even mate with immature spiders (Biaggio et al. 2016). These behaviors may have evolved to minimize female mate choice or reduce the risk of pre-mating sexual cannibalism. Other strategies like coercive or direct mounting of females in the absence of courtship may have evolved in response to competition from rival males in the area as well as to override female mate choice (Johns et al. 2009; Wilgers et al. 2009; De Young & Wilgers 2016). Females may choose males using a courtship threshold rule whereby they accept any male that exceeds a specified level of courtship. If females use a courtship threshold rule for choosing males and don't discriminate the source of the courtship, non-courting males may be able to successfully parasitize the courtship displays of accepted males by mounting the female while another male courts. This pattern has been documented in the Australian redback spider (*Latrodectus hasselti* Thorell, 1870) (Stoltz & Andrade 2010). Among these examples of opportunistic mating strategies, males are able to successfully mate without courting. Male spiders may be able to adaptively combine strategies that minimize cannibalism with strategies that minimize courtship by approaching distracted females already copulating with a male, directly mount the female, and achieve inseminations concurrently with the first male. Although this behavior is possible given the paired structure of male and female genitalia, it has been poorly documented in the spider literature. Here I describe natural occurrences of this behavior in a single species and also discuss the possible adaptive advantages to males.

In the fall of 2015, three separate observations of two male *Rabidosa punctulata* (Hentz, 1844) (Araneae: Lycosidae) simultaneously mating with a single female were found under natural field conditions. All observations occurred within a three week time frame between October 3rd and October 24th. Spiders were serendipitously discovered while hand-collecting other species of wolf spider by headlamp. All three mating triads were observed between 1850 and 2300h and occurred on short lawn grass in the front yard of my house in Selinsgrove, Snyder County, Pennsylvania, USA. During the first two observed simultaneous double matings, both males were

discovered already mounted on the female. Mounting time lasted for 43 minutes and 61 minutes respectively for these mating triads between the time of discovery until one of the males dismounted. In both mating triads, males shifted positions until they were each able to access at least one spermatheca. Although simultaneous insertions from different males were not directly observed in either of the first two triads, alternating leg spine erection among both males was observed in very close succession and over several minutes suggesting that alternating hematodochal expansion was occurring for each male. This pattern was verified in the third triad observation.

During the initial observation of the third triad, I was able to observe a courting male begin mounting a female within a few minutes while a second male was approaching the same female within a few centimeters away. The second male did not court and also mounted the female within one minute of the first male's mount. I then left for about four minutes to retrieve my camera and a plastic lid from my house and returned to the mating spiders. I noted the time again and positioned the plastic lid under the mating triad and transported all three to my dining room table for photographing (see Figs. 1A–E). Neither male dismounted the female during transport nor did they shift position. I then placed the triad inside a large plastic sweater box (59.7 cm x 47.9 cm x 14.9 cm) for continuous observations and macrophotography. None of the three spiders were touched nor were they contacted except with placement of the lid under the triad. All photos were the result of 3–6 focus-stacked images using Zerene® Stacker software. This allowed increased depth of field and better documentation of leg positions and pedipalps for both males. Photos were taken using a Canon® 6D and MPE-65mm 1-5x macro lens with a Canon® MT-24EX macro twin lite flash mounted to the lens. I was able to individually identify the males because the second mounted male (B) had one leg II missing. Mounting time of both males persisted for at least four hours. The first male (A) mounted the female at approximately 1901h with the second male (B) initiating a mount less than one minute later. Both males were still mounted four hours later at 2301h when direct observation ceased. I returned at 0500h the following morning to find both males dismounted and no cannibalism evident. An additional leg was missing from male B however, suggesting some aggression. All three spiders were returned to the field that morning.

In all three mating triads, the spiders spent most of the observation time in one of three positions with the remainder of the time
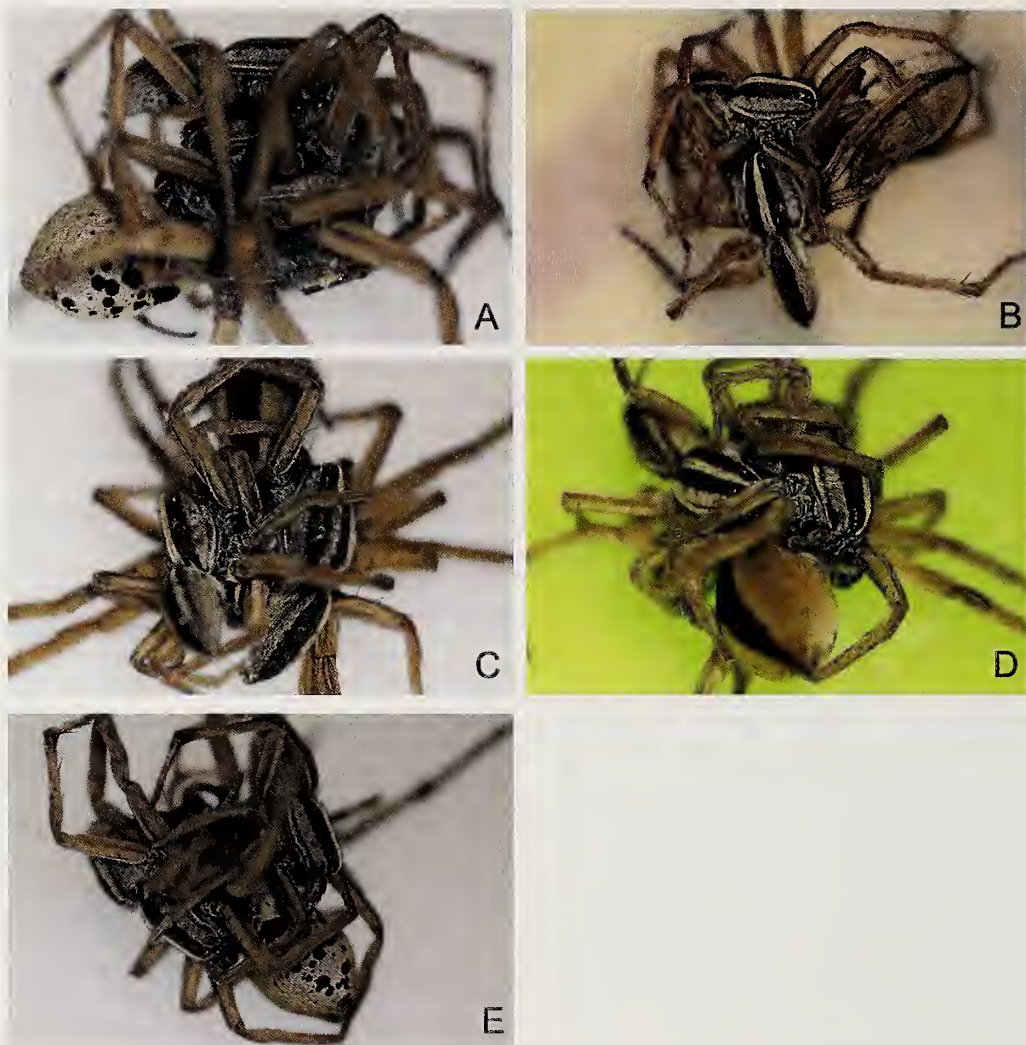
Figure 1.—Simultaneous double mating in the wolf spider *Rabidosa punctulata* (Lycosidae). A. Position one with the second male mounted dorsally on the first male in an anterior-to-posterior direction. The top male scraped his pedipalp along the lateral side of the second male's abdomen, presumably in a failed attempt to locate the epigynum (9.6% of total copulation time). B. Position two with one male oriented at 90 degrees relative to the other male. Both males are in a ventral-ventral position relative to the female with the female inverted on her dorsum (12.1% of total copulation time). C–E. Position three with both males in an anterior-posterior position relative to the female but displaced laterally (75.4% of total copulation time). C. Dorsal view with both males' legs intertwined to maintain position. D. Female rotating abdomen to accommodate one of the male's pedipalps. E. Ventral view with one of the male's pedipalps nearly inserted while the other male waits for the female to rotate her abdomen the other direction for his turn at insertion.

transitioning between these positions. The percentage of time mounted in each of these positions was documented only for the third mating triad and is described below.

For position one, one male is mounted dorsally but in an anterior-to-posterior direction with respect to the other male, which in turn, is mounted anterior-to-posterior dorsally on the female (Fig. 1A). While in this position, the dorsal-most male exhibited palpal moistening and palpal scraping along the antero-lateral portion of the lower male's abdomen, apparently trying to locate the epigynum (Fig. 1A). At the same time, the male immediately dorsal to the female was able to engage in palpal insertions. This orientation occurred with both males at various times occupying the lower position closer to the female. This position comprised only 23 minutes (9.6%) of the total observed mounting time.

Position two was observed with each male oriented at a 90 degree angle from one another in a ventral-ventral position relative to the female. In this position, the female was inverted on her dorsum (Fig. 1B) and the male oriented closest to the long axis of the female was

able to achieve insertions. The other male oriented at 90 degrees was unable to gain an insertion but did interfere with the first male's insertion with repeated palpal scraping at the other male's palps. This resulted in several partial hematodochal expansions of the first male's palp outside of the female's reproductive tract. It was unclear if these pedipalp sparrings were the incidental by-product of one male's failed insertion attempts or a direct attempt at copulatory interference. This position accounted for 29 minutes of the observed mounting time (12.1%).

The third position made up the majority of the observation period (181 minutes or 75.4% of mounting time) with the remaining seven minutes (2.9% of mounting time) consisting of males in transition between one of these three positions. Position three consisted of both males in an anterior-to-posterior position dorsally on the female and resembled a typical Type III mating position for lycosids (Foelix 1996) with the exception that both males were somewhat displaced laterally (Figs. 1C–D). While in this position, both males were able to successfully insert pedipalps into the female alternately as the female

rotated her abdomen to one side or the other but only with one of their pedipalps. This frequently required both males to move to a lateral position on the female to accommodate an insertion (Fig. 1E, ventral view). While in the third position, the males never switched sides of the female during the entire observation period. No overt aggression was witnessed during the entire four-hour observation period.

Prior to these observations, I had never seen even single copulating pairs of *R. punctulata* in the field. It was therefore surprising that three observations occurred within a short period and all of them involved mating triads of *R. punctulata*. This suggests that simultaneous matings of *R. punctulata* may be particularly common in the field for at least this population of the species. Although spider densities were not exhaustively sampled, over twenty individuals occurred over a 6 m² area within the yard when the third mating triad was observed. Minimum density estimates were not noted for the first two triad observations but all three mating triads occurred within 21 days of each other and in the same general area so presumably had similar population densities. It is possible that these events are the result of unusually high *R. punctulata* densities and therefore illustrate an atypical behavior. A recent laboratory study with experimental triads of two male *R. punctulata* paired with a single unmated female failed to result in simultaneous matings (De Young & Wilgers 2016) suggesting that either laboratory matings may underestimate the occurrence of this behavior or that different environmental conditions are necessary to promote this particular alternative mating strategy. De Young & Wilgers (2016) found that males tended to be successful with a direct mount rather than courtship strategy when two males were interacting with a single female and both males showed some mating behavior. Mountings of the first male were disrupted in 50% of cases by the second male but no simultaneous matings were reported where both males remained on the female.

There are several potential adaptive advantages to mounting an already-mating female compared to mating first, or waiting until a male dismounts and then attempting to mount. First, courtship may be unnecessary when approaching a copulating female (Wilgers et al. 2009). Since many wolf spider courtship displays can attract predators (Pruden & Uetz 2004; Hoefler et al. 2008; Roberts & Uetz 2008; Fowler-Finn & Hebets 2011a, b; Wilgers et al. 2014; Clark et al. 2016) males may minimize their own predation risk while another male courts. If female *R. punctulata* don't discriminate the source of the courtship as in the Australian redback spider and females follow a threshold courtship rule for mating (Stoltz & Andrade 2010), then the opportunity cost of choosing to not court a female may be small. Second, wolf spider courtship is energetically expensive to males (Kotiaho et al. 1998; Hoefler et al. 2008; Cady et al. 2011) allowing courtship parasitizing males to conserve energy for copulation itself or for seeking additional females. Third, males that wait until a pair are mating may suffer reduced pre-mating cannibalism compared to males that are first to initiate courtship or choose to mate with recently mated females. Mating females may be distracted, cataleptic, or immobilized by the male. In this way, copulating females may be functionally equivalent to a feeding or recently molted female that shows little or no aggression toward males (Jackson 1986; Prenter et al. 1994; Fromhage & Schneider 2005). In some cases, mating males may actually induce a quiescent state in the female, making cannibalism less likely (Becker et al. 2005). Previous studies show that mated female wolf spiders exhibit significantly higher pre-mating cannibalism frequencies than unmated females (Persons & Uetz 2005) indicating that if secondary males wait until the first male dismounts, sexual cannibalism risk will increase. A fourth related benefit includes reduced risk of being rejected by an already mated female. For some wolf spider species, females tend toward monandry and have low frequencies of second matings even if they don't cannibalize (Norton & Uetz 2005; Persons & Uetz 2005; Jiao et al. 2011). A fifth benefit of simultaneous mounting may include a reduced chance of direct

agonistic interaction between males since non-mating male wolf spiders may spar and grapple with each other, especially in the presence of an unmated female (Aspey 1977a, b; Delaney et al. 2007; Hoefler et al. 2009). Finally, male mounting of copulating females may eliminate the need for aggressive tactics toward the female which may injure both the female and potentially result in the death of the male (Johns et al. 2009; Wilgers et al. 2009)

Despite these adaptive benefits of simultaneous mating, there are several apparent costs. Male-male interference that could reduce sperm transfer and increase the energetic costs of mating are likely. In my direct observations, males showed palpal sparring, missed insertions, attempts at pedipalp insertions into the other male's venter, and attempts to displace the other male during insemination. Collectively, these inefficiencies may lead to prolonged matings. Longer copulation is associated with increased risk of post-mating cannibalism among some wolf spiders (Wilder & Rypstra 2007) and may make spiders more susceptible to other predators. Stratton et al. (1996) reported maximum copulation durations for *Rabidosa hentzi* (Banks, 1904) and *Rabidosa rabida* (Walekenaer, 1837) at 25.67 minutes and 90 minutes respectively. Our minimum copulation duration of four hours for one of the observed triads suggests that simultaneous matings extend copulation duration. For many lycosids, prolonged copulation may serve as a form of mate guarding to reduce sperm competition while the sperm capacitates (Stratton et al. 1996; Szirányi et al. 2005). Such mate-guarding functions of prolonged mating would be ineffective under conditions of simultaneous mating. In *R. punctulata*, direct mounting without courtship is condition-dependent, with larger, better condition males adopting this strategy more frequently than small poor-condition males (Wilgers et al. 2009). My observations suggest that non-coercive direct mounting of copulating females may make direct mounts far less risky for poor-condition males.

Collectively these observations have important implications for our understanding of the strength of intersexual selection acting on male courtship displays, the nature of male-male competitive interactions before and during mating, sperm competition, and cryptic female mate choice. These observations also expand on the diverse repertoire of opportunistic mating tactics exhibited by male spiders. Most intriguing, secondary mounting strategies can result in male mating success without courtship, female acceptance, or overt coercive behaviors toward females, all while reducing pre-mating cannibalism risk. If so, then males that are at a competitive disadvantage through either courtship or aggressive mating tactics may engage in this strategy to achieve some fitness benefits. Although not directly observed here, courtship parasitism of attractive males or parasitism of other male subjugation of females may be a viable strategy for improved mating success for less competitive males. Additional research on the taxonomic occurrence, frequency, and social context of this behavior is needed.

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SHORT COMMUNICATION

The first case of gynandry in Mygalomorphae:
Pterinochilus murinus, morphology and comments on sexual behavior (Araneae: Theraphosidae)

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Abstract. A bilateral gynandromorph specimen of the tarantula *Pterinochilus murinus* Pocock, 1897 is here described and illustrated. In addition, encounters with conspecific females were studied. The possible explanations for this case of gynandry are discussed. This constitutes the first formal report of gynandry in a mygalomorph spider.

Keywords: Bilateral-gynandromorph, mygalomorph

Gynandromorph spiders are adult individuals in which female and male parts of the body are discretely combined. These combinations can occur laterally, transversely, obliquely or irregularly. Intersexuality is another condition in which parts of the body have an intermediate morphology between sexes (Roberts & Parker 1973). After Blackwall (1867), about 50 cases of gynandry have been reported for araneomorph spiders (Cokendolpher & Sissom 1988) comprising the families: Agelenidae (Kaston 1961), Dictynidae (Kaston 1961), Gnaphosidae (Roberts & Parker 1973), Hahniidae (Kaston 1961), Linyphiidae (Hackman 1950-1951; Knülle 1954; Waaler 1970; Roberts & Parker 1973; Palmgren 1979; Wunderlich 1994), Liocranidae (Krumpalova 1999), Lycosidae (Holm 1941; Wiebes 1959; Kaston 1961; Mackie 1969; Gack & Helversen 1976; Stratton 1995), Oxyopidae (Simó et al. 2007), Philodromidae (Roberts & Parker 1973), Phrurolithidae (Kaston 1961), Salticidae (Kaston 1961; Roberts & Parker 1973), Theridiidae (Roberts & Parker 1973; Kumada 1989) and Thomisidae (Kaston 1961). Despite this diversity of findings, the frequency of gynandromorphs in spiders is estimated to occur in one out of 17,000 individuals (Palmgren 1979), thus being a rare phenomenon. As far as we know, there are no formal reports of gynandry among Mygalomorphae, however at least an image of the theraphosid *Poecilotheria* sp. gynandromorph is available on the Internet at http://arachnoboards.com/threads/poecilotheria-ornata-gynandromoph.60259/

In this paper, we report the first gynandromorph tarantula, describe its morphology and study its sexual behavior in encounters with adult conspecific females.

Spiderlings of *Pterinochilus murinus* Pocock, 1897 were donated from an amateur tarantula keeper (Montevideo, Uruguay) and raised in the laboratory to maturity. After adulthood, a couple of them copulated in the laboratory in January 2010, the female laid the egg sac and spiderlings emerged in April 2010. Some of these spiderlings were raised and they reach maturity in December 2014. Among these individuals, we found one with gynandromorphic characteristics which we studied and describe here.

Measurements are in mm and were obtained using a stereomicroscope (Olympus, SZH, Japan) with ocular micrometer. Drawings were done with a camera lucida (Olympus, SZH, Japan). Spination nomenclature follows Pérez-Miles et al. (2008). The gynandromorph individual was compared with the descriptions of females and males of the species, given by Gallon (2002, 2008) and deposited in the Arachnological Collection of the Facultad de Ciencias, Montevideo, Uruguay (FCE-My 1406). Abbreviations: AME = anterior median eyes, ALE = anterior lateral eyes, PME = posterior median eyes, PLE

= posterior lateral eyes, OQ = ocular quadrangle (including lateral eyes); d = dorsal, p = prolateral, r = retrolateral; PMS = posterior median spinnerets, PLS = posterior lateral spinnerets; v = ventral; ri = right, le = left.

The gynandromorph presents bilateral differences the right side shows female characteristics and the left side male ones (Fig. 1) which corresponds to the regular Type I gynandromorph of Roberts & Parker (1973). Female and male areas were normally developed after maturation, but in the first post-maturation molt, the male palpal organ was aberrant (Fig. 2A, B) while the female palp remains normal (Fig. 2D, E). Total length, excluding chelicerae and spinnerets, is 37.51. Carapace length 15.38, width 13.13. Anterior eye row procurved, posterior straight. Eye sizes: AME 0.6 (ri, le), ALE 0.8 (ri, le), PME 0.6 (ri, le), PLE 0.8 (ri, le). OQ length 2.4, width 3.1, clypeus 0.7. Fovea transverse, straight, width 1.5. Labium length 1.7, width 2.2 with 56 cuspules, maxillae with 245 (ri)–254 (le) euspules. Sternum length 8.8, width 6.5. Chelicerae proximal segment 8.38 (ri), 7.25 (le), distal segment 6.25 (ri), 5.25 (le). Chelicerae teeth on the promargin (distal–basal): 9 large and 7 small (ri); 2 large, 4 small, 2 large, 8 small (le). Tarsi I–IV densely scopulated, scopula I–IV entire (ri more dense and dark than le). Metatarsi I and II scopulate; III scopulate on their apical 3/4 (ri), 2/3 (le), IV scopulate on their apical 1/2 (ri), 2/3 (le). Tibia I (le) with a distal proventral apophyses (Fig. 2C). Tibia I (ri) normal (Fig. 2F). Flexion of metatarsus (le) retrolateral with respect to tibial apophyses. Palpal organ (le) as in Fig. 2A, B. Length of leg and palpal segments (right–left side; femur/ patella/ tibia/ metatarsus/ tarsus). Leg I: 13.25–14.25/ 8.13–7.75/ 10.63–11.63/ 9.63–10.63/ 6.63–6.88. Leg II: 12.13–12.88/ 7.13–7.13/ 9.25–10.00/ 8.88–9.75/ 6.25–6.50. Leg III: 10.63–11.00/ 6.38–5.75/ 7.38–8.25/ 9.00–10.38/ 6.25–6.25. Leg IV: 13.00–12.88/ 6.63–6.75/ 7.75–10.63/ 12.50–13.50/ 6.75–7.00. Palp: 9.38–8.50/ 6.00–5.63/ 6.75–6.88/ — / 7.75–3.38. Spination: Femora (ri, le) I–IV and palp 0. Patellae (ri, le) I–IV and palp 0. Tibiae (ri) I 1p; II 0; III 1p; IV 1p; palp 1p; (le) I 0; II 1p; III 1p; IV 1p, 1r; palp 1p. Metatarsi (ri) I 0; II 0; III 3v, 1p, 1r; IV 2v, 1r; (le) I 0; II 0; III 3v,3p; IV 4v, 2d. Tarsi I–IV and palp 0. Abdomen (ri) densely covered by reddish-brown setae, and spermatheca with one curved tubuliform receptacle with globose fundus (Fig. 2G); (le) sparsely covered with setae. Spinnerets PLS (ri) basal segment 1.15; medial 0.95, apical 1.35; (le) basal segment 1.07, medial 0.83, apical 1.11. PMS (ri) 2.26, (le) 1.95.

Color in life (Fig. 1): (ri) Cephalothorax, abdomen and legs covered by reddish-brown setae with darker radial lines on carapace and darker transverse lines on abdomen; (le) Cephalothorax, abdomen and legs dark brown, with reddish brown setae on femora; radial reddish brown lines on carapace.

Figure 1.—Gynandromorph of *Pterinochilus murinus*. Habitus, dorsal view, scale: 200 mm.
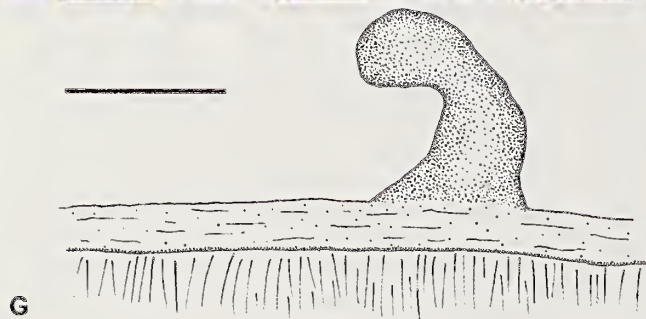
The gynandromorph matured in December 2014, after that it molted again in April 2015. After the maturation molt, the gynandromorph presented the palpal organ with the usual morphology of the species but after the last molt the palpal organ showed an aberrant morphology (Fig. 2A, B). The tibial spur after both molts presented a morphology typical of the species but lacking the apical spine (Fig. 2C).

The individuals were raised in plastic containers (14 × 14 x 6 cm) in early instars and then transferred to glass containers (25 × 15 x 15 cm). They were fed with *Drosophila* sp. and *Musca* sp. (Diptera) in early instars, and then *Blaptica dubia* (Blattaria, Blaberidae) according with the individual size of the spider. During rearing, the mean temperature was 17.5 °C (range 11.9–22.7) and mean relative humidity 75% (range 68–85). Behavioral observations were conducted in the laboratory in glass arenas (25 × 12 x 18 cm). The gynandromorph was placed carefully in the container of a female, as far as possible from her. The behavior of the couple was registered by video recording and notes. The observations started when the gynandromorph contacted the female web or substratum and finished if no courtship was observed in 15 minutes or if the female attacked. Four encounters of the gynandromorph with different females were performed with an interval of 48 hours between trials. During the experimental period mean temperature was 25°C (range 24–26) and mean relative humidity 60% (range 47–67).

The gynandromorph did not perform courtship in any of the four encounters with females and females did not show any sexual response. In one trial a female attacked the gynandromorph and damaged leg I.

A common explanation of gynandromorphy in animals (e.g., *Drosophila*) involves the non-disjunction of X chromosomes early in

Figure 2.—Gynandromorph of *Pterinochilus murinus*. A. Left palpal organ, ventral view. B. Same, prolateral view. C. Left tibial apophysis, prolateral view. D. Right palp, ventral view. E. Same, prolateral view. F. Right tibia, prolateral view. G. Right spermatheca, dorsal view. Scales: 1 mm.

development (White 1973; Stratton 1995). Presumably, the same mechanism explains the gynandry in spiders, although it was not investigated in this group (White 1973). Kaston (1961) suggested that gynandromorphy would be less frequent in spiders due to the chromosomal system, where females have two or more sexual X chromosomes than males. The occurrence of a gynandromorph from a female zygote would imply the loss of two or more chromosomes. If we assume this explanation, the regular lateral Type I gynandromorph we found could be the result of the loss of at least one pair of X chromosomes in the first cleavage of the zygote, in a genetically determined female, as suggested by Roberts and Parker (1973). From the morphological point of view, each half of the gynandromorph correspond with the normal body development of each sex comparing with the descriptions of *P. murinus* (Gallon 2002, 2008). A slight difference was found in the spermathecal morphology of the gynandromorph and could be interpreted as an individual variation which is in accord with intraspecific variation (Gallon 2002, 2008). However, from the physiological point of view, female hormonal factors seemed to predominate considering the spider molted after the male half reach adulthood. Also, the gynandromorph was rejected in the encounters with other females probably because of its female behavioral characteristics. These facts could reinforce the hypothesis of Roberts & Parker (1973) who proposed that this type of gynandromorph is produced from a genetically determined female. The lower diversity and scarce studies on Mygalomorphae in comparison with Araneomorphae, could explain our unusual finding.

ACKNOWLEDGMENTS

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SHORT COMMUNICATION

A vertebrate-eating jumping spider (Araneae: Salticidae) from Florida, USA

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Abstract. The salticid spider *Phidippus regius* C.L. Koch, 1846 is documented preying on small frogs (*Hyla* spp., *Osteopilus septentrionalis*) and lizards (*Anolis carolinensis* and *Anolis sagrei*) in Florida, USA. Female as well as male *P. regius* were engaged in feeding on this type of vertebrate prey. A total of eight incidents of *P. regius* devouring vertebrates have been witnessed in seven Florida counties. Furthermore, we report an incident of a large unidentified *Phidippus* sp. (possibly *P. bidentatus* F. O. Pickard-Cambridge, 1901) preying on an immature anole lizard in Costa Rica. *P. regius*, otherwise known to feed almost exclusively on insects and spiders, is one of the world's largest salticid spiders reaching a maximum recorded body length of 2.2 cm. Most other salticid spiders appear to be too small in body size to overcome vertebrate prey. Vertebrate predation by salticid spiders has not been previously documented in the scientific literature. Together with Salticidae, spiders from 27 of 114 families (24%) are currently known to occasionally consume vertebrate prey.

Keywords: Generalist predators, predation, prey, Dactyloidae, Hylidae, Southeastern USA

With >5,900 described species, the jumping spider family (Salticidae, a member of the clade Dionycha) is the largest spider family, composing ≈13% of the total spider species (World Spider Catalog 2017). Accordingly, the spider species in this family exhibit an enormous diversity of life styles and foraging strategies (Edwards et al. 1974; Ross 2008; Meehan et al. 2009; Pekár & Toft 2015; Nyffeler et al. 2016). More than a decade ago, Jackson et al. (2005) discovered an East African salticid (*Evarcha culicivora* Wesolowska & Jackson, 2003) which was imbibing vertebrate blood after piercing the bodies of female mosquitoes which recently had a blood meal. The report of this first jumping spider feeding on vertebrate blood was considered a major discovery by the arachnological scientific community. But not only are there salticids that indirectly feed on vertebrate blood after piercing the bodies of blood-fed mosquitoes, there are also spiders from this family that directly feed on vertebrate prey. An incident of this type was witnessed in the early 1990s in the Gainesville area in northern peninsular Florida, USA. There, two former wildlife graduate students witnessed a large salticid spider (*Phidippus regius* C.L. Koch, 1846) in the process of devouring a small tree frog. These students mentioned their observation of a frog-eating jumping spider as a side note during the '1995 North American Amphibian Monitoring Program Conference' in Toronto, Canada (O'Neill & Boughton 1996), but they did not regard this as something spectacular since they were not aware of the fact that vertebrate-eating was a behavioral trait at that time unknown for jumping spiders. Actually, feeding on small frogs and lizards by spiders in Florida is not uncommon, but this refers to non-salticid spiders (e.g., Goin 1943; Owen & Johnson 1997; Jeffery et al. 2004).

We decided to conduct an internet survey to find out how widespread vertebrate predation by salticids might be. This was achieved by searching for reports using Google Search, Google Scholar, Google Books, and Google Pictures as well as the Thomson-Reuters and Scopus databases. During our search we found six additional reports of predation on small vertebrates by salticids, all of which had been posted more recently on social media sites along with photographic evidence. Some of the bloggers who had posted reports were contacted by us to obtain additional information on their observations. In addition, there was another incident that was reported directly to one of the authors (GBE) but not posted. Photographs were deposited in the digital archives of the Division of Herpetology (UF-Herpetology), Florida Museum of Natural History, University of Florida. In the following we give an account of the eight predation events (Table 1 and Fig. 1), followed by a discussion of the phenomenon of vertebrate predation by salticids.

Phidippus regius is one of the world's largest salticids reaching a maximum recorded body length of 2.2 cm in adult females and 1.8 cm in adult males. Large females have a leg span approaching 3.5 cm, and the largest males may have a leg span exceeding 4 cm, although they have less body mass than females. The front legs have enlarged setae (macrosetae, often erroneously referred to as 'spines') on the ventral surface that assist with prey capture, and of course, like most spiders, they have a venomous bite. These spiders reach an adult female body mass of ≈0.5–1 g (Anderson et al. 1979; Anderson 1990). They are stout, heavy-bodied, powerful spiders that are capable of jumping more than their body length straight up a vertical surface to catch prey (Edwards 1980). They have been previously observed to catch invertebrate prey (predominantly insects and spiders) much larger than themselves, including such relatively dangerous prey as adult shieldback katydids twice the length of the spider (GBE, pers. obs.). They live in old field habitats and often frequent small palms and palmettoes (≈1.0–3.0 m height) as adults, where females nest inside the newest fully developed but unopened leaf (Edwards 1990). From these protected nests, the females can forage on the trunk and other leaves, and onto adjacent shrubs or small trees if available.

Phidippus regius captured frogs with a body length of ≈1–1.5 times the spider's length and lizards with a snout-vent length of ≈1.5–2.5 times the spider's length (Table 1). Some salticids are known to capture prey organisms up to three times their own length and twice their own mass (Robinson & Valerio 1977). No mass measurements of vertebrate prey of *P. regius* are available. But based on the predator/prey size ratios (Table 1) we can deduce from literature data that the frogs (≈1.5–3 cm body length) devoured by *P. regius* had an estimated mass of ≈0.375–2 g (comp. frog body mass data by Stuckert et al. 2009; Costa-Pereira et al. 2010), whereas the captured
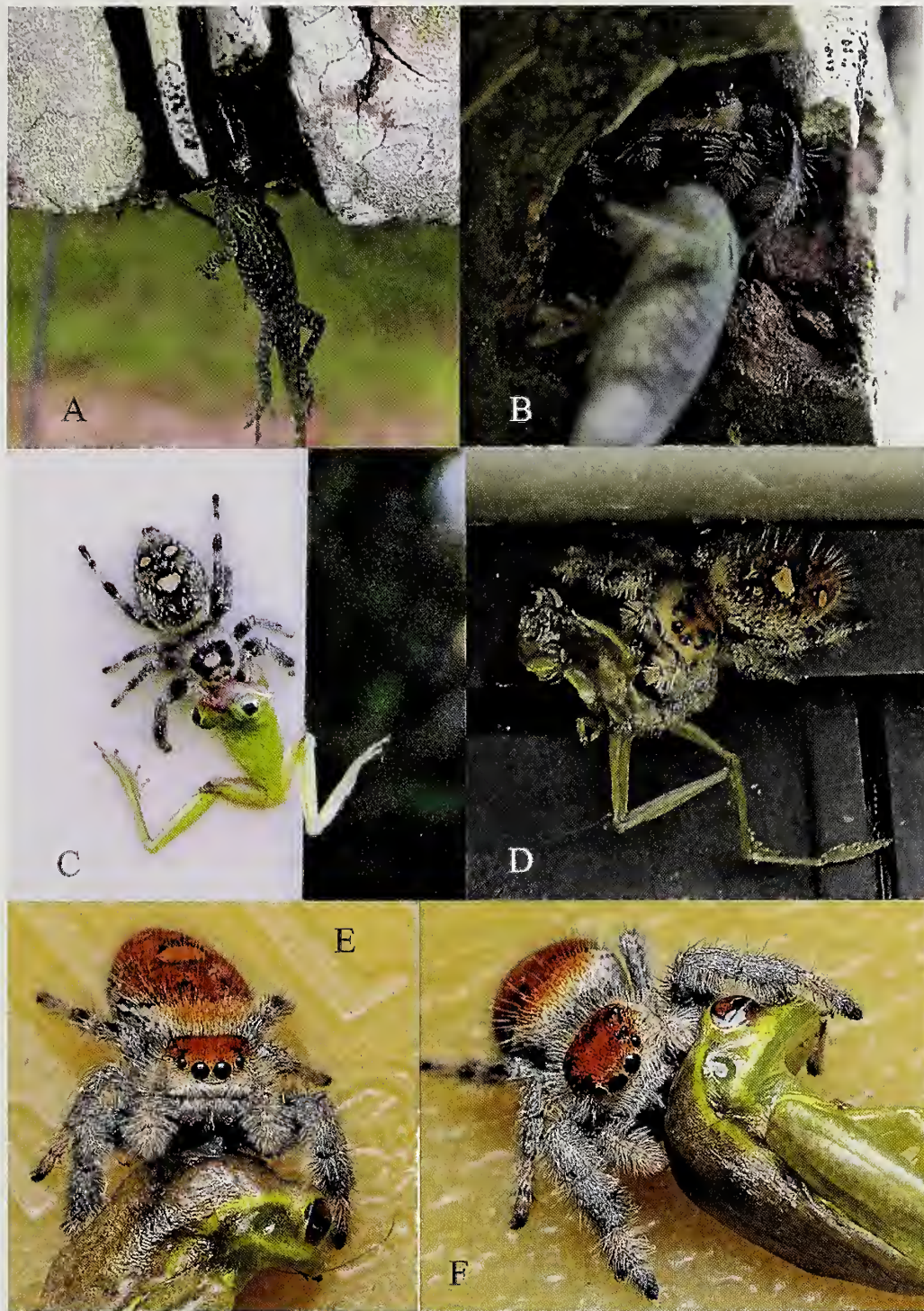
Figure 1.—A. & B. Female *Phidippus regius* consuming a Caroline anole (*Anolis carolinensis*) in the Sunnyhill Restoration Area, Florida (photographs by Jeff Hollenbeck; UF-Herpetology 179622); C. Female *Phidippus regius* feeding on a Cuban tree frog (*Osteopilus septentrionalis*) in a residential area in Land O'Lakes, Florida (photograph by Jeanine DeNisco; UF-Herpetology 179483); D. Female *Phidippus regius* eating a juvenile Cuban tree frog (*Osteopilus septentrionalis*) in a rural area in Holopaw, Florida. The spider was found sitting in the water filtration system attached to a well (photograph by Loret Setters; UF-Herpetology 179623). E. & F. Female *Phidippus regius* eating a Cuban tree frog (*Osteopilus septentrionalis*) in Lake Placid, Florida (photograph by Martin Fisher; UF-Herpetology 179625).

lizards (≈2.3–5 cm snout-vent length) had an estimated mass of ≈0.5–3 g (comp. lizard body mass data by Meiri 2010; Senczuk et al. 2014). Most species of salticid spiders are probably too small – with a body mass of only 0.006–0.2 g (Greenstone & Bennett 1980; Anderson 1990)—to be able to overpower such large vertebrate prey.

However, it is highly likely that apart from *P. regius*, other large salticid species occasionally prey on small vertebrates. An incident of this type was witnessed in 1993 on the peninsular region of Puntarenas, Costa Rica, referring to a large unidentified *Phidippus* sp. devouring an immature anole lizard (Stanislav Macik, pers.

Table 1.—Eight incidents of predation on small vertebrates by the jumping spider *Phidippus regius* in Florida, USA. Length estimates (cm) used to calculate predator/prey ratios are based on photographs. Predator length = total body length, excluding legs. Prey length = snout-vent length.

Location	Habitat type	Spider predator	Prey species	Predator/prey length ratio	Source
Alachua County: Gainesville area	Pine flatwoods site	*P. regius* Large individual	*Hyla femoralis* (Hylidae)	N/A	O'Neill & Boughton (1996)
Escambia County:	Spider resting on concrete block wall	*P. regius* Male	Unident. (Hylidae)	N/A	*
Hillsborough County: Tampa	Planted plot of saw palmetto (*Serenoa repens*)	*P. regius* Male	*Hyla squirella* (Hylidae)	1 : 1	Scott Pittenger (pers. comm.)
Marion County: Umatilla Sunnyhill Restoration Area	Restoration area; spider sitting in a crack in a fence.	*P. regius* Female	*Anolis carolinensis* (Dactyloidae)	1 : 2.5	Jeff Hollenbeck (pers. comm.)
Pasco County: Land O'Lakes	Backyard adjacent to a conservation area; spider resting on a fence	*P. regius* Female	*Osteopilus septentrionalis* (Hylidae)	1 : 1	Jeanine DeNisco (pers. comm.)
Osceola County: Holopaw	Garden; spider found sitting on water filtration system attached to a well	*P. regius* Female	*Osteopilus septentrionalis* (Hylidae)	1 : 1	Loret Setters (pers. comm.)
Highlands County: Lake Placid	Ranch; spider was dragging a frog across an old plastic container	*P. regius* Female	*Osteopilus septentrionalis* (Hylidae)	1 : 1.5	Martin Fisher (pers. comm.)
N/A, Florida	N/A	*P. regius* Male	*Anolis sagrei* (Dactyloidae)	1 : 1.5	**

* A photo sent to one of the authors (GBE) by email has been inadvertently erased
** Photo posted on the 'etsi' website is no longer available at http://img2.etsystatic.com/006/0/6684602/il_fullxfull.374244

comm.). This was likely *P. bidentatus* F. O. Pickard-Cambridge, 1901, the largest known species in Central America with a maximum recorded female body length of about 1.7 cm (Eric Olson, pers. comm.; Edwards 2004). Moreover it is conceivable that large salticids such as *Hyllus* spp. in Africa and Asia, approximately the same size as *Phidippus regius*, are capable of catching small frogs or lizards.

Spiders, by and large, are generalist predators that utilize a large number of different prey categories (Riechert & Harp 1987). "Being a generalist predator has some advantages in that there are always different prey choices around" (Moore 2015). If one of their staple prey gets scarce, generalist predators can switch to alternative prey. A generalist feeding behavior is particularly advantageous in the case of cursorial spiders (e.g., *Phidippus* spp.) known to feed infrequently in the field (see Jackson 1977; Dean et al. 1987; Young 1989; Nyffeler 1999). *Phidippus regius* with its ability to occasionally capture small vertebrate prey in addition to its usual invertebrate prey, is a typical example of a predator with a generalist feeding behavior and the exceedingly broad feeding niche of this spider is presumed to improve its survival.

A literature survey conducted by us revealed that spiders from 26 families (i.e., Actinopodidae, Agelenidae, Anyphaenidae, Araneidae, Barychelidae, Clubionidae, Corinnidae, Ctenidae, Ctenizidae, Cybaeidae, Cyrtaucheniidae, Desidae, Dipluridae, Gnaphosidae, Hexathelidae, Idiopidae, Liocranidae, Lycosidae, Nephilidae, Pholcidae, Pisauridae, Sparassidae, Tetragnathidae, Theraphosidae, Theridiidae, and Trechaleidae) have been reported so far to include vertebrates in their diets (compare Butler & Main 1959; Vollrath 1978; McCormick & Polis 1982; Brunet 1998; Gopi Sundar 1998; Menin et al. 2005; Toledo 2005; Wehtje 2007; Pertel et al. 2010; Almeida-Reinoso & Coloma 2012; Brooks 2012; Ficetola et al. 2012; Nyffeler & Knörnschild 2013; Rojas-Morales & Escobar-Lasso 2013; Nyffeler & Pusey 2014; Australian Museum 2016; Kerr-Dineen 2016). Together with the Salticidae reported in this paper, spiders from 27 out of 114 families (= 24% of all families) are currently known to occasionally consume vertebrate prey.

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SHORT COMMUNICATION

Spiders feeding on earthworms revisited: consumption of giant earthworms in the tropics

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Abstract. Predation on earthworms is common in some generalist predator species, as for example several ground beetle species (Coleoptera: Carabidae) that frequently feed on earthworms. In spiders (Araneae), however, such behavior is far less well documented. A survey of reports on spiders feeding on earthworms yielded a total of 44 naturally occurring predation events. Spiders from 14 families were observed feeding on earthworms in nature, and species from two additional families consumed earthworm prey in captivity. Earthworm predation by spiders has been observed in temperate, subtropical, and tropical regions in 18 different countries. Tropical spiders from the families Theraphosidae (Mygalomorphae) and Ctenidae (Araneomorphae) accounted for 59% of the reported predation events. Reports from French Guiana document the capture of giant earthworms (0.6–1 m in length) by the giant tarantula, *Theraphosa blondi* (Latreille, 1804). Predation on giant earthworms by large tarantulas has also been observed in rainforest habitats in Brazil, Ecuador, Peru, and Venezuela. Wandering spiders (Ctenidae) are known to feed on earthworms in Belize, Brazil, Costa Rica, French Guiana, Guyana, and Singapore. Quite obviously, larger-sized mygalomorph and araneomorph spiders in humid tropical rainforests are predators with broad feeding niches—including earthworms and vertebrate prey in addition to arthropod prey—and this is presumed to improve the survival of these spiders. By comparison, reports of earthworm predation in temperate climate are rarer, and recent molecular studies of the diet composition of lycosid and linyphiid spider species in Swedish arable fields suggest that earthworms are not a common prey of these species.

Keywords: Oligochaeta, Ctenidae, Theraphosidae, generalist predators, diet composition

Predation on earthworms is common in some generalist predator species, as for example several ground beetle species (Coleoptera: Carabidae) that frequently feed on earthworms (King et al. 2010). In spiders (Araneae), however, such behavior is far less well documented, as spiders are predominately predators of arthropods, with insects by far being the most dominant prey group (Nyffeler 1999; Birkhofer & Wolters 2012; Pekár & Toft 2015). In addition, some spider species are known to occasionally include small vertebrates in their diets (McCormick & Polis 1982; Henschel 1994; Menin et al. 2005; Nyffeler & Knörnschild 2013; de Carvalho et al. 2016; Nyffeler et al. 2017). Still other spiders use plant food to supplement their diets (Nyffeler et al. 2016). It has further been documented that some spider species also feed on unusual prey such as slugs, snails, velvet worms, polychaete worms, woodlice, amphipods, shrimps, crayfish, or freshwater crabs (McLay & Hayward 1987; Nyffeler & Symondson 2001; Nyffeler & Pusey 2014; Bhukal et al. 2015; Pekár & Toft 2015; Franco & Monge-Nájera 2016). According to a previous assessment by Nyffeler et al. (2001), spiders from eight families also feed on earthworms under natural conditions.

Over the last 15 years, new evidence of earthworm predation by spider species has been published in the scientific literature or on the internet. Here we update a previous assessment (Nyffeler et al. 2001) and discuss the overall evidence for earthworm predation in spiders to provide a more realistic view on the commonness and taxonomic or geographic patterns. We define earthworms as oligochaete worms represented by several families which live in the litter layer or in soil, and feed on dead organic matter (Lal 2006).

An extensive bibliographic search was conducted to identify all published reports on predation on earthworms by spiders using the ISI Web of Science Thomson-Reuters database, Scopus database, Google Scholar, Google Books, and Google Pictures. Social media sites were also searched for content indicating earthworm predation by spiders. Furthermore, inquiries among biologists were undertaken for unpublished reports on this topic. In total, we found 44 reports of predation on earthworms by spiders, about half of which had previously been published in the scientific literature (Appendix 1).

Overall, spiders from 14 families (Agelenidae, Atypidae, Ctenidae, Hexathelidae, Lycosidae, Pisauridae, Salticidae, Segestriidae, Sicariidae, Sparassidae, Tetragnathidae, Theraphosidae, Theridiidae, Thomisidae) have been reported to feed on earthworms in nature, and individuals from two more families (Amaurobiidae, Araneidae) accepted earthworm prey in captivity (Appendix 1). It should be noted that the majority (>75%) of reported cases of predation on earthworms refers to spiders that forage as active hunters without the use of a catching web (see Fig. 1A for an exception).

So far, predation on earthworms has been reported from all continents except Africa and Antarctica (Appendix 1). This type of predator-prey interaction occurs in temperate, subtropical, and tropical climates. In the temperate regions of Europe and North America, earthworms consumed by spiders usually are members of the family Lumbricidae (e.g., Fig. 1B). A case of this trophic interaction was filmed in a park in Amsterdam, Netherlands. This video documents a crab spider (*Xysticus ulmi* (Hahn, 1831)) killing an earthworm about 13 times longer than itself (online at https://www. youtube.com/watch?v=a9lf3tKw1E0). In another instance, a small linyphiid spider (*Macrargus rufus* (Wider, 1834)) was seen feeding on a potworm (Enchytraeidae) in a forest habitat in Belgium (Rudy Jocqué, pers. comm.). This incident is not included in our assessment (Appendix 1), since potworms are not regarded as earthworms in the strict sense (see Sims & Gerard 1985). However, due to the close taxonomic relationship between potworms and earthworms (both
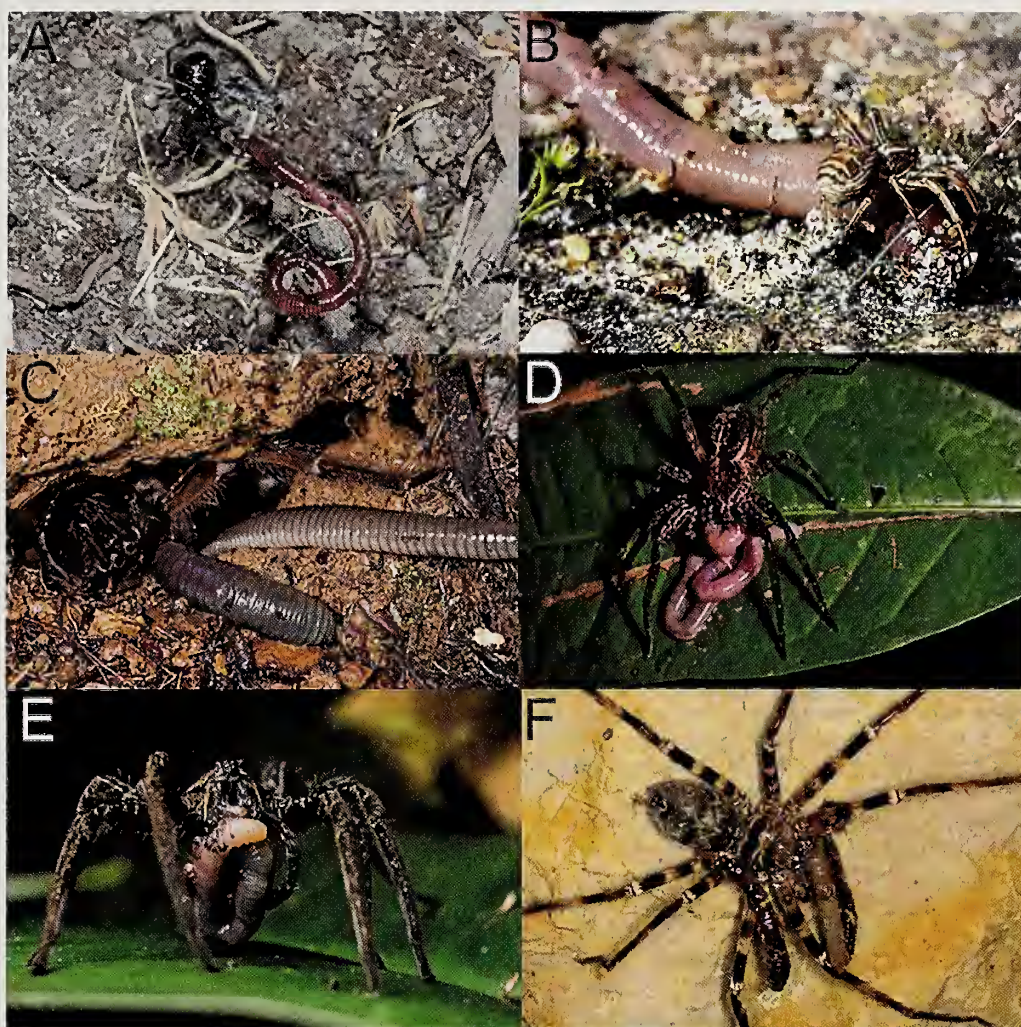
Figure 1.—Photographic evidence of earthworm predation by spiders. A. Redback spider (*Latrodectus hasselti*) with unidentified earthworm prey in its web in a garden in Melbourne, Australia (photo by Iain Duncan). B. Crab spider *Xysticus ulmi* killing a lumbricid earthworm in a park in Amsterdam, Netherlands (Copyright: Sammy Jean Cunze, itsVision.tv). C. *Theraphosa blondi* pulling a captured giant earthworm (presumably *Rhinodrilus* sp.) into its burrow in rainforest in French Guiana (photo by C.E. Timothy Paine). D. *Ctenus* sp. feeding on an earthworm in rainforest in Guyana (photo by Andrew Snyder). E. Ctenid spider feeding on an unidentified earthworm in rainforest in Belize (photo by Anton Sorokin). F. *Heteropoda maxima* feeding on an unidentified earthworm near Ban Koenphavang, Laos (photo by Peter Jäger).

being oligochaete worms with similar *modus vivendi*), we feel that this case should be mentioned in this review.

In the northern part of South America, large fossorial tarantulas have been observed catching and consuming giant earthworms (Appendix 1). A very impressive predation event was observed in a tropical rainforest at the Nouragues Biological Station, French Guiana. A giant tarantula, *Theraphosa blondi* (Latreille, 1804), was recorded feeding on a giant earthworm of 50–60 cm length. The spider was sitting in its burrow and half of the earthworm was pulled into the burrow while the other half was visible (W. Lapinski, pers. obs.). Another impressive scene was observed in the same area near Nouragues. Here, a *T. blondi* individual was seen slowly pulling a giant earthworm of approx. 1 m length into its burrow (Fig. 1C; C.E. Timothy Paine, pers. comm.). Paine stated "….The earthworm was stretched across a trail through the rainforest, grasping (as best he could) to anything for friction. The tarantula was pulling the earthworm from about ⅓ of the way along the body. So the earthworm was trying to cling to the soil with both ends. It was stretched taut…." The way the giant earthworm was pulled into the burrow resembles the way snakes are captured and pulled into

burrows by this tarantula species (Rick West, pers. comm.; Gordon 1993). The observer watched the scene for about 10 minutes, took photos (Fig. 1C), and left. When he returned to the scene about one hour later, spider and earthworm had disappeared. Although it has not been documented that the spider actually killed and ate the earthworm, it can be strongly suspected that the spider devoured the earthworm out of human sight after pulling it into its burrow (C.E. Timothy Paine, pers. comm.). According to Rick West (pers. comm.) at least half a dozen theraphosid spider interactions with earthworms witnessed in tropical rainforests have all been predation events where the earthworm was dragged into the spider's burrow, killed, and consumed. Based on photos (Fig. 1C) the captured giant earthworm most likely belonged to a species in the genus *Rhinodrilus*, placed in the family Rhinodrilidae by some taxonomists (Tomas Pavlíček, pers. comm.; Csaba Csuzdi, pers. comm.) and in the family Glossoscolecidae by other taxonomists (Emmanuel Lapied, pers. comm.). In another instance, a *T. blondi* individual consumed an earthworm of 30 cm length near Manaus, Brazil (Nyffeler et al. 2001). Other incidents of *T. blondi* consuming earthworms occurred in the Kaw Mountains, French Guiana (Rick West, pers. comm.) and in Guyana (Lewis

2014). Furthermore, large tarantulas – *Theraphosa apophysis* (Tinter, 1991) and *Megaphobema velvetosoma* Schmidt, 1995 – were witnessed feeding on giant earthworms in Venezuela, Ecuador, and Peru (Rick West, pers. comm.).

A second group of tropical rainforest spiders frequently consuming earthworms are wandering spiders from the family Ctenidae (Fig. 1D & E; Andrew Snyder, pers. obs.). The earthworm depicted in Fig. 1D most likely belongs to a species placed in the family Rhinodrilidae (Csaba Csuzdi, pers. comm.; Tomas Pavlíček, pers. comm.) or Glossoscolecidae (Emmanuel Lapied, pers. comm.). Such incidents of ctenids consuming earthworms have been observed and photographed in Belize, Brazil, Costa Rica, French Guiana, Guyana, and Singapore (Appendix 1). *Ctenus* spp. in particular are abundant inhabitants of tropical rainforests (Portela et al. 2013). They are mainly nocturnal spiders that forage by ambushing prey on the leaf litter and low understory vegetation (Almeida et al. 2000; Salvestrini & Gasnier 2001; Gasnier et al. 2002). These medium-sized to large spiders may have 0.7–2.2 g body mass and are capable of catching prey ranging from 0.5–1.5 g (Lapinski & Tschapka 2013, 2014). A giant huntsman spider (*Heteropoda maxima* Jäger, 2001; Sparassidae) with a legspan of approx. 20 cm has also been observed feeding on an earthworm in Laos (Fig. 1F; Peter Jäger, pers. comm.). This spider species typically lives near cave entrances (Jäger 2001).

Large tarantulas such as *T. blondi* reach body weights of 25–65 g, with a maximum of up to >100 g (Saul-Gershenz 1996; Zachariah et al. 2007; Smith 2008). Such heavy spider species have high energy requirements and can be expected to feed on large prey organisms such as small vertebrate species (McCormick & Polis 1982; Menin et al. 2005). A giant earthworm, which can reach a body weight of >100 g (Moreno & Paoletti 2004), is a very profitable prey item for these giant tarantulas. Furthermore, giant earthworms are high quality prey characterized by high protein content (Moreno & Paoletti 2004) and may not pose a large risk to predators due to their limited defense abilities. The observation of spiders feeding on giant earthworms is generally important, as little is known about the natural enemies of giant earthworms (e.g., O'Donnell et al. 2005; Strüssmann et al. 2013).

Tropical spiders from the families Theraphosidae and Ctenidae accounted for 59% of the reported incidents of earthworm predation by spiders. Spiders from these families are typical generalist predators with broad feeding niches (Brunet 1998; Lapinski & Tschapka 2013; Lewis 2014; de Carvalho et al. 2016; Rick West, pers. comm.). The fact that they capture a large variety of different prey types including arthropods, vertebrates and even earthworms is presumed to improve their survival in humid tropical rainforests (also see Nyffeler et al. 2017). In temperate regions, predator-prey interactions between spiders and earthworms seem to be less common, as indicated by recent molecular tests on lycosid and linyphiid species in arable fields in Sweden (Roubinet et al. 2017). On the other hand, temperate region earthworms (Oligochaeta: Lumbricidae) can compose a substantial portion (up to almost 50%) in the diets of arachnids of the genera *Leiobunum* C.L. Koch, 1839 and *Hadrobunus* Banks, 1900 (Opiliones: Sclerosomatidae) (Halaj & Cady 2000); such earthworm-eating harvestmen, however, are facultative predators getting access to earthworm prey rather by scavenging.

ACKNOWLEDGMENTS

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Appendix 1.—Records of spiders feeding on earthworms (44 records from the field and 3 records from spiders in captivity).

Spider taxonomic classification	Country	Type of evidence	Source
MYGALOMORPHAE			
Theraphosidae			
Theraphosa apophysis (Tinter, 1991)	Venezuela	Field observation	Rick West (pers. comm.)
	Venezuela	Field observation	Rick West (pers. comm.)
	Venezuela	Field observation	Rick West (pers. comm.)
	Venezuela	Field observation	Rick West (pers. comm.)
Theraphosa blondi (Latreille, 1804)	Brazil	Field observation	Nyffeler et al. (2001)
	French Guiana	Photo	C.E. Timothy Paine (pers. comm.)
	French Guiana	Field observation	Rick West (pers. comm.)
	French Guiana	Field observation	Witold Lapinski (pers. obs.)
	Guyana	Field observation	Lewis (2014)
	N. South America	Photo	Francesco Tomasinelli[A]
Megaphobema velvetosoma Schmidt, 1995	Peru	Field observation	Rick West (pers. comm.)
	Ecuador	Field observation	Rick West (pers. comm.)
Atypidae			
Atypus affinis Eichwald, 1830	United Kingdom	Field observation	Savory (1926)
Hexathelidae			
Hadronyche versuta (Rainbow, 1914)	Australia	Field observation	Brunet (1998)
ARANEOMORPHAE			
Agelenidae			
Eratigena atrica (C.L. Koch, 1843)	Switzerland	Field observation	Nyffeler et al. (2001)
Tegenaria sp.	France	Field observation	Nyffeler et al. (2001)
Amaurobiidae			
Amaurobius fenestralis (Stroem, 1768)	In captivity	Observation in captivity	Nyffeler et al. (2001)
Amaurobius ferox (Walckenaer, 1830)	In captivity	Observation in captivity	Nyffeler et al. (2001)
Araneidae			
Araneus diadematus Clerck, 1757	In captivity	Observation in captivity	Nyffeler et al. (2001)
Ctenidae			
Ancylometes rufus (Walckenaer, 1837)	Brazil	Field observation	Nyffeler et al. (2001)
Ancylometes sp.	Belize	Field observation	Anton Sorokin (pers. comm.)
Ctenus amphora Mello-Leitão, 1930	Brazil	Field observation	Thierry Gasnier (pers. comm.)
	Brazil	Field observation	Thierry Gasnier (pers. comm.)
Ctenus crulsi Mello-Leitão, 1930	Brazil	Field observation	Thierry Gasnier (pers. comm.)
	Brazil	Field observation	Thierry Gasnier (pers. comm.)
	Brazil	Field observation	Thierry Gasnier (pers. comm.)
Ctenus curvipes (Keyserling, 1881)	Costa Rica	Photo	Philipp Ric. Figueroa[B]
Ctenus sp.	Guyana	Photo	Andrew Snyder (pers. obs.)
	French Guiana	Photo	Anonymous[C]
	Singapore	Photo	Christopher Teo[D]
	Singapore	Photo	Adrian Cho[E]
	South East Asia	Photo	Joseph Ang[F]
	South East Asia	Photo	Eldie Aaron Justim[G]
Lycosidae			
Pardosa sp.	USA	Field observation	Vogel (1971)
Trochosa terricola Thorell, 1856	France	Field observation	Nyffeler et al. (2001)
Unknown	USA (Pennsylvania)	Field observation	Darryl Moran[H]
	USA (Michigan)	Field observation	Anonymous[I]
Pisauridae			
Dolomedes sp.	Germany	Video	Peter Blendowski[J]
Salticidae			
Platycryptus undatus (De Geer, 1778)	USA (Michigan)	Field observation	Ross (2008)
Segestriidae			
Segestria florentina (Rossi, 1790)	France	Field observation	Nyffeler et al. (2001)
Sicariidae			
Loxosceles intermedia Mello-Leitão, 1934	Brazil	Prey found in web	Fischer et al. (2006)
Sparassidae			
Heteropoda maxima Jäger, 2001	Laos	Photo	Peter Jäger (pers. comm.)
Tetragnathidae			
Meta menardi (Latreille, 1804)	United Kingdom	Prey found in web	Smithers (1996)
Theridiidae			
Latrodectus hasselti Thorell, 1870	Australia	Photo	Iain Duncan (pers. comm.)

Appendix 1.—Continued.

Spider taxonomic classification	Country	Type of evidence	Source
Thomisidae			
Xysticus ulmi (Hahn, 1831)	Netherlands	Video	Dagmar Cunze[K]
Xysticus sp.	Switzerland	Field observation	Nyffeler (1982)
	Switzerland	Field observation	Nyffeler et al. (2001)

[A] Francesco Tomasinelli: online at http://www.arkive.org/goliath-bird-eating-spider/theraphosa-blondi/image-G112597.html

[B] Philipp Ric. Figueroa: online at https://www.flickr.com/photos/80335180@N06/16578886018

[C] Anonymous : online at https://www.flickr.com/photos/60313790@N08/5539782616

[D] Christopher Teo: online at https://www.flickr.com/photos/snapperholic/3372187502/in/photolist-68ZkRQ

[E] Adrian Cho: online at https://www.flickr.com/photos/adriancho/3351350135

[F] Joseph Ang: online at https://www.flickr.com/photos/parampita2009/3351265515/in/album-72157612958642434/

[G] Eldie Aaron Justim: online at https://www.shutterstock.com/de/pic-432352723/stock-photo-wandering-spider-with-a-worm-prey.html?src=2sqDzsMDQZEOGel9dmvmig-1-23

[H] Darryl Moran: online at https://www.flickr.com/photos/dwmoran/5880202140/in/photostream/

[I] Anonymous: online at http://i.imgur.com/FNYHAG7.jpg

[J] Peter Blendowski: online at https://www.youtube.com/watch?v=a89RyuDstus

[K] Dagmar Cunze: online at: https://www.youtube.com/watch?v=a9lf3tKw1E0

SHORT COMMUNICATION

Decreases in the size of riparian orb webs along an urbanization gradient

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Abstract. Urbanization is associated with a variety of anthropogenic impacts that alter aquatic ecosystems and could affect riparian web-spinning spiders. The objective of this study was to evaluate how changes in web structural features and body condition of a horizontal orb-weaver are associated with surrounding levels of urbanization. Along an urban watershed in Puerto Rico, we found a significant negative relationship between the capture area of webs and in the body condition of spiders with increasing levels of surrounding impervious surface. We propose that these changes in web structure and body condition are associated with variations in the diversity and quality of prey, as well as the loss of riparian substrate in more heavily urban areas.

Keywords: Capture area, Puerto Rico, Río Piedras watershed, *Tetragnatha*, web structure

Increasing levels of urbanization and their associated environmental stressors can have strong effects on the diversity, morphology, and behavior of organisms (Miyashita et al. 1998; Prosser et al. 2006; McKinney 2008). Web-spinning spiders are commonly found along the riparian areas of streams where they can take advantage of emerging aquatic insects (Burdon & Harding 2008; Greenwood & McIntosh 2008; Lambeets et al. 2008; Chan et al. 2009; Akamatsu & Toda 2011). Common impacts of human activities within urban watersheds include the loss of riparian vegetation and changes in aquatic insect diversity (Ramírez et al. 2012). Changes in substrate availability (Vollrath et al. 1997; Laeser et al. 2005; Chan et al. 2009) and changes in the diversity and quality of prey (Sherman 1994; Schneider & Vollrath 1998; Mayntz & Toft 2001; Davis et al. 2011) have both been shown to affect the web-spinning behaviors and overall fitness of spiders. Body condition indices have been found to be a useful method in monitoring changes in nutrition and growth and are associated with an individual's overall fitness (Jakob et al. 1996). Changes in web-spinning behaviors have been analyzed by measuring variations in the structures of orb web produced under different conditions (Zschokke & Vollrath 1995; Vollrath et al. 1997; Schneider & Vollrath 1998; Hesselberg & Vollrath 2004).

Despite limited web variation within a species, some individual spider characteristics (e.g., size, sex, and weight) (Heiling & Herberstein 2000), along with a number of abiotic (e.g., temperature, humidity and wind) (Vollrath et al. 1997) and biotic (e.g., prey diversity and abundance) (Sherman 1994; Blackledge & Zevenbergen 2006) factors are known to contribute to variability in the structural features of orb webs. Web design patterns are a behavioral blueprint or phenotype, which gets expressed daily (Vollrath et al. 1997; Toscani et al. 2012) and represents a major energetic output for spiders (Sherman 1994; Heiling & Herberstein 1999). A typical orb web is a spiral of sticky silk overlaying a radial array of threads in a frame with lines attached to a type of substrate (Vollrath 1988). Previous studies that have investigated the effects of urbanization on spider taxa have generally only focused on changes in diversity or abundance (Laeser et al. 2005; Prieto-Benítez & Méndez 2011; Horvath et al. 2014), but with practically no information on changes in web structures or body condition.

We focused on a species of horizontal orb-weaving spider, *Tetragnatha boydi* O. Pickard-Cambridge, 1898 (Araneae: Tetragnathidae), which is distributed throughout Africa, South Asia, Central and South America and the Caribbean (Okuma 1992). Being an aquatic ecosystem specialist and because of its high abundance along the Río Piedras watershed, *T. boydi* makes an ideal species for this study. The objective of this study was to assess if urbanization has an effect on *T. boydi* resulting in changes in their web structures and body condition. Along the urban gradient of the Río Piedras watershed, increasing levels of urbanization results in the loss of riparian vegetation and a decrease in aquatic insect richness, with the loss of sensitive taxa (e.g. Ephemeroptera and Trichoptera) but a large increase in the abundance of resilient taxa (e.g. Chironomidae) (de Jesús-Crespo & Ramírez 2011). Small chironomid midges feed primarily on fine particulate organic matter (FPOM), which has been shown to be a low quality food source limited in phosphorus and nitrogen when compared to periphyton (Cross et al. 2003), which is consumed by larger ephemeropterans in less urbanized parts of the watershed. Due to the strong effects that substrate and prey availability have on web-spinning behaviors (Sherman 1994; Vollrath et al. 1997; Zschokke 1997; Schneider & Vollrath 1998), we predicted that the size of the web would decrease in more urban areas where there is less riparian vegetation and only small, low quality chironomids available as prey.

This study was conducted in the Río Piedras watershed, in the metropolitan area of San Juan, Puerto Rico. The drainage area is about 49 km^2 and it originates at around 150 m asl and runs for 16 km (Ramírez et al. 2014). The watershed is highly urbanized with heavily modified channels and has been classified by the U.S. Environmental Protection Agency (EPA) as highly polluted (Lugo et al. 2011). Six sites, representing six subwatersheds, were chosen for this study (Appendix 1). These six sampling sites are part of the San Juan Urban Long Term Research Area (ULTRA) project since 2009 and land use data are available for each subwatershed (Ramírez et al. 2014). Using the percent of impervious surface for each subwatershed, we established a gradient from Site 1, the least urbanized with 12.9 percent impervious surface, to Site 6, the most urbanized with 77.2 percent impervious surface (Appendix 1). The percent of impervious surface is strongly correlated with changes in water physicochemistry in the Río Piedras watershed (e.g., increasing concentrations of chloride, sulfate, sodium, magnesium and higher levels of specific conductivity) (Ramírez et al. 2014). These physicochemical variables are generally associated with increasing levels of urbanization (Walsh et al. 2005; Lugo et al. 2011; Ramírez et al. 2014), which makes the gradient ideal for assessing possible effects of urbanization.

A 100 meter transect was established at each of the six sites along the watershed. Transects were surveyed at night (~1900–2000) for

Table 1.—Pair-wise comparisons of Pearson correlation coefficients for the seven web parameters. A total of 56 webs was analyzed (df=54). Significant differences of p ≤ 0.05 indicated by (*).

	capture area	free zone	mesh size	radii length	number of radii	spiral length
free zone	0.49*	-	-	-	-	-
mesh size	0.49*	0.43*	-	-	-	-
radii length	0.88*	0.50*	0.45*	-	-	-
number of radii	-0.09	-0.09	-0.21	0.29*	-	-
spiral length	0.94*	0.45*	0.35*	0.01	0.01	-
number of spirals	0.69*	0.24	0	0.24	0.24	0.80*

webs of adult female *T. boydi* and around ten individual webs were sampled at each site (Appendix 2). Spider size and ontogeny have been shown to have an effect on web-spinning behaviors and, therefore, we controlled for this factor by utilizing only adult females. Webs that were incomplete, had large gaps or holes, and those in the middle of construction were not utilized. Webs were powdered with cornstarch to make them visible and a photo was taken of the web and a centimeter grid paper (for the purpose of scale; Eberhard 1976) using a digital camera (Pentax Optio WG-3). Individuals corresponding to each web were collected and the body condition of each spider was determined by calculating a ratio index using the equation: mass/cephalothorax width^3 (Jakob et al. 1996). Cephalothorax width was chosen to represent body size due to previous studies finding this to be the most accurate estimator of overall body mass in spiders (Hagstrum 1971; Marshall & Gittleman 1994; Jakob et al. 1996) and by raising cephalothorax width to the third power we take into consideration that linear measurements generally increase isometrically with mass (Jakob et al. 1996). Cephalothorax width was measured by photographing each spider and using image processing software Image J (Abramoff et al. 2004). After being photographed, spiders were placed in a drying oven at 70°C for a minimum of 48 hours in order to obtain their dry mass.

Various web features are known to reflect neurological and environmental impacts on spiders (Samu & Vollrath 1992; Sherman 1994; Vollrath et al. 1997; Hesselberg & Vollrath 2004; Wyman et al. 2011). We measured: number of radii and radii length, number of spirals and spiral length, capture area, mesh size, and free zone area. Mesh size was calculated using the formula suggested by Herberstein and Tso (2000):

$$mesh\,size = \frac{1}{2}\left(\frac{r_u - Hr_u}{s_u - 1} + \frac{r_l - Hr_l}{s_l - 1}\right)$$

Where r_u and r_l are the distances in centimeters from the center of the web to the outermost capture spiral threads for the upper and lower vertical sectors, Hr_u and Hr_l refer to the distance in centimeters from the center of the web to the innermost capture spiral thread of the upper and lower vertical sector, and s_u and s_l refer to the number of capture spiral threads in the upper and lower sectors (Herberstein & Tso 2000). When the vertical sector had missing parts, large holes, or any other factor that impeded calculating an accurate estimate, the horizontal sectors were considered to the right or left side of the hub. If neither vertical nor horizontal sectors could be used, a sector was arbitrarily chosen and a note was made.

The statistical program R (R Core Team 2012) was used to verify that all data met the statistical requirements for parametric tests, along with all other subsequent statistical analyses. Utilizing calculated pair-wise Pearson correlation coefficients, we tested the seven web variables for collinearity. When a significant correlation ($P \leq 0.05$) and a Pearson correlation coefficient greater than 0.65 was found we selected and kept the variable we determined to most accurately predict changes in web structures (Esselman & Allan 2010). All webs and spiders were pooled together to calculate mean values for each site (Appendix 2). Linear regression analyses were utilized to

test for relationships between averages of the selected web variables for each site and the percent impervious surface of each site's subwatershed. Regression analyses were also conducted to test for relationships between the mean values of the body condition ratio index for each site with the percent impervious surface for each subwatershed.

We found a large degree of variation among the seven web parameters along the urban gradient but not all were found to have a significant relationship with the surrounding percentage of impervious surface in each subwatershed. A complete summary of the data for web parameter measurements and body condition index for each site can be found in Appendix 2. Number of radii was more or less constant among sites and linear regression analyses showed no significant relationships between percent impervious surface with mean number of radii ($n = 6$, $R^2 = 0.32$, $P = 0.24$). Mesh size and free zone area decreased slightly in more urban sites but there was no significant relationship between percent of impervious surface with mesh size ($n = 6$, $R^2 = 0.26$, $P = 0.30$) or free zone area ($n = 6$, $R^2 = 0.39$, $P = 0.18$). Radii length, spiral length, number of spirals and capture area were significantly correlated with each other (Table 1) and all had a significant negative relationship with increasing levels of impervious surface. Mean radii length was found to decrease by around 48% along the urban gradient from Site 1 to 6 ($n = 6$, $R^2 = 0.74$, $P = 0.03$). The change in mean spiral length was even more pronounced with Site 6 being around 65% shorter than Site 1 ($n = 6$, $R^2 = 0.80$, $P = 0.02$) and the mean number of spirals decreased by around 36% between Site 1 and Site 6 ($n = 6$, $R^2 = 0.85$, $P = 0.01$). The greatest difference was seen in mean capture area, which decreased by 76% between Site 1 and Site 6 ($n = 6$, $R^2 = 0.74$, $P = 0.03$). As these four variables (radii length, spiral length, number of spirals and capture area) were all significantly correlated with each other (Table 1) and all are associated with general web size, capture area was selected as the variable which best represents overall changes in web structures along the urban gradient (Fig. 1a). Similar to the web parameters mentioned, the mean ratio index representing body condition of the spiders at each site was also found to decrease by around 40% from Site 1 to Site 6 and it had a significant negative relationship with the percent impervious surface ($n = 6$, $R^2 = 0.67$, $P = 0.05$) (Fig. 1b).

As predicted, the web size and body condition of *T. boydi* had significant negative relationships with increasing levels of urbanization. We attribute the alterations in web structures and body condition to be indirectly associated with increasing levels of anthropogenic impact to the stream ecosystem along the urban gradient. Previous studies have shown that small chironomid midges dominate aquatic insect communities in more urbanized areas of the Río Piedras watershed (de Jesús-Crespo & Ramírez 2011) and this could result in smaller webs due to the reliance on a low quality food source of small prey (Pasquet et al. 1994; Sandoval 1994; Schneider & Vollrath 1998). In addition, there is also less riparian vegetation and substrate available in more urbanized sites which could lead to smaller webs due to changes in microclimate and availability of attachment points for orb-weaving spiders (Vollrath et al. 1997).
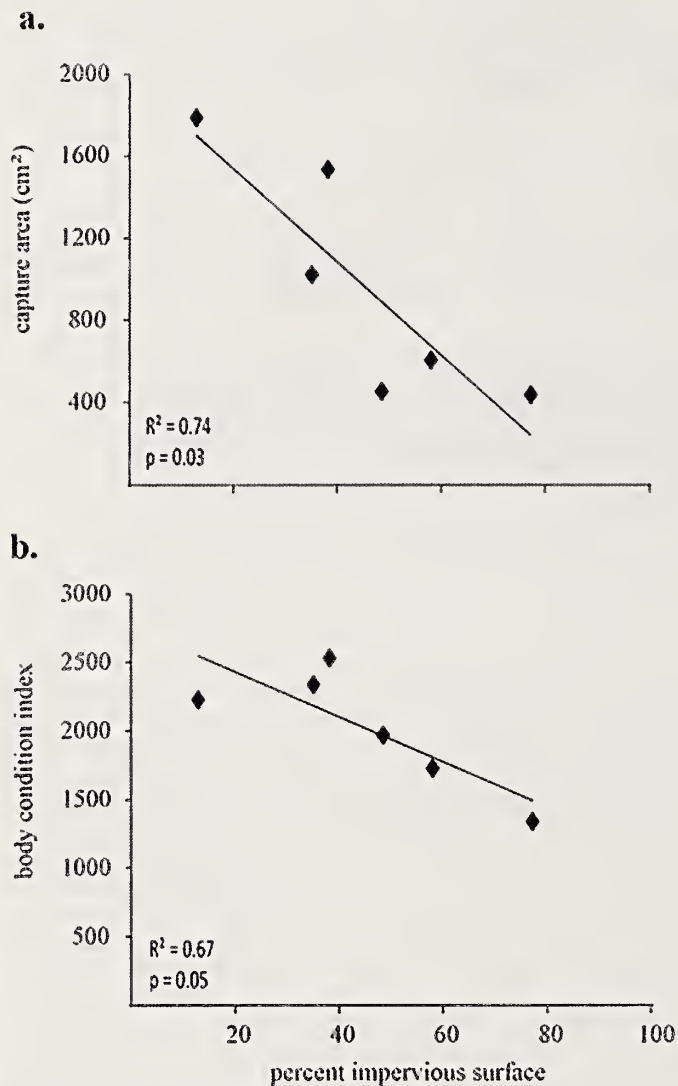
a.

b.

Figure 1a-b.—Linear regression analyses. a. mean capture area and b. mean body condition index with the percent impervious surface for each site within the six subwatersheds. Regression analyses with p ≤ 0.05 were considered significant.

The majority of the studies investigating changes in web structures due to abiotic and biotic factors have been conducted for only a small number of taxa, mainly in the family Araneidae (Sandoval 1994; Sherman 1994; Vollrath et al. 1997). The most commonly recorded web parameters for these studies were web area, thread length and mesh size. Sherman (1994) found that *Larinioides cornutus* (Clerck, 1757) increased their web area and total thread length when prey was limited but there was no significant change in mesh size. Sandoval (1994) found that *Parawixia bistriata* (Rengger, 1836) spun larger webs with increased mesh size when larger prey episodically became more abundant. Under controlled lab conditions, Vollrath et al. (1997) found that *Araneus diadematus* Clerck, 1757 alter their webs in response to changes in abiotic factors such as temperature and humidity. Decreases in temperature resulted in similar web areas, but larger mesh sizes; while a decrease in humidity resulted in smaller capture areas, shorter spiral lengths, and smaller mesh sizes (Vollrath et al. 1997). Substrate is another important factor because spiders would have to alter web dimensions in relation to available attachment points and when confined to smaller areas, orb-weaving spiders were found to spin significantly smaller webs (Vollrath et al.

1997). Our results of a tetragnathid species in response to urbanization showed significant changes in capture area and length of spirals, but not in mesh size. However, due to differences in how web structures were measured and with possibly inherent differences in behavior between taxa, it may be difficult in comparing results from the few studies conducted up to this point and different species of orb-weaving spiders may respond differently to similar stimuli.

Apart from obvious changes in web-spinning behavior due to differences in body size and ontogeny (Eberhard 1988), or changes in behavior induced by the presence of harmful toxins (Samu & Vollrath 1992; Hesselberg & Vollrath 2004), it may be difficult to determine the underlying reasons for changes in web structures. One overall trend however is that it appears that capture area and total spiral length are web structures commonly adapted to both abiotic and biotic factors, while other web parameters such as free zone area, and number of radii appear to be more static. In conclusion, we found that the web structures and body condition of riparian tetragnathids can be significantly altered by increasing levels of urbanization that impact stream ecosystems. Within a heavily urbanized watershed riparian, tetragnathids are most likely being affected, at least in some part, by some combination of several factors. Therefore, future studies focused on how particular mechanisms affect *T. boydi* web-spinning behaviors would be beneficial in determining how specific factors associated with urbanization are impacting these important riparian consumers within urban watersheds. As urban areas continue to dominate the landscape, it is becoming increasingly important to understand how the behavior and fitness of organisms is being affected by these anthropogenic activities.

ACKNOWLEDGMENTS

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Site #	% IS
1	12.9
2	35.1
3	38.2
4	48.5
5	58.0
6	77.2
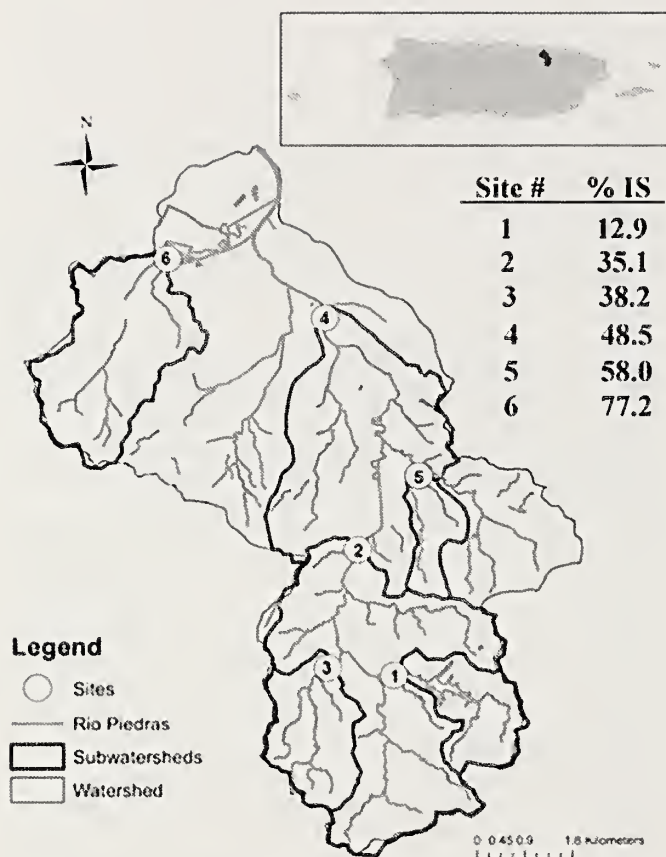
Appendix 1.—Study sites within the Río Piedras watershed. Site 1 is the least urbanized with the lowest amount of surrounding percent impervious surface (%IS) and Site 6 the most urbanized with the greatest amount of surrounding percent impervious surface

Appendix 2.—Mean ± standard deviation for the seven web parameters and spider body condition (ratio index = mass/cephalothorax width^3) at each site (Site 1-6), along with the percent of impervious surface (%IS) for each site's sub-watershed and the number of webs and individual spiders measured at each site (n), with the exception s of Site 1 (only 8 spiders measured) and Site 6 (10 spiders measured).

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
	12.9 %IS	35.1 %IS	38.2 %IS	48.5 %IS	58.0 %IS	77.2 %IS
	(n = 10)	(n = 10)	(n = 9)	(n = 10)	(n = 10)	(n = 7)
Web parameters						
capture area (cm^2)	1783 ± 720	1019 ± 695	1532 ± 1144	452 ± 180	601 ± 339	434 ± 330
freezone area (cm^2)	33.0 ± 11.0	31.9 ± 13.1	49.4 ± 25.6	18.0 ± 4.0	20.0 ± 7.4	14.5 ± 9.9
mesh size (cm)	0.69 ± 0.18	0.70 ± 0.26	0.86 ± 0.17	0.42 ± 0.08	0.55 ± 0.15	0.55 ± 0.16
number of radii	11.5 ± 1.8	12.9 ± 1.9	11.8 ± 2.8	12.4 ± 1.4	12.8 ± 2.0	12.4 ± 1.5
number of spirals	27.4 ± 4.6	20.6 ± 7.5	21.4 ± 7.4	20.2 ± 5.8	19.8 ± 5.2	17.4 ± 2.9
radii length (cm)	283 ± 79	225 ± 99	253 ± 90	144 ± 45	181 ± 66	148 ± 67
spiral length (cm)	2700 ± 869	1690 ± 847	1960 ± 1075	941 ± 347	1108 ± 508	951 ± 576
Spider body condition						
ratio index	2226 ± 660	2337 ± 519	2530 ± 841	1967 ± 374	1724 ± 720	1337 ± 351

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